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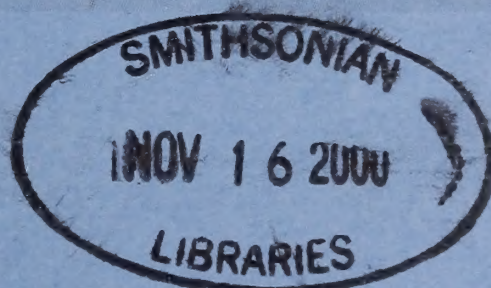
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Cover Illustration: Predation by the opossum *Didelphis marsupialis* on the rattlesnake *Crotalus durissus*. A photograph taken by Almeida-Santos, Antniazzi, Sant'Anna, and Jared.

Predation by the Opossum *Didelphis marsupialis* on the Rattlesnake *Crotalus durissus*

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Abstract: Opossums are considered natural predators of snakes and possess resistance to the venom of some viperids. The resistance of *Didelphis* to *Crotalus* venom has been demonstrated through biochemical and immunological assays. However, systematic observations on the behavior of adult *Didelphis* preying on venomous snakes have never been conducted. In this study the predatory and defensive behaviors of *Didelphis marsupialis* and *Crotalus durissus*, respectively, were analyzed in captivity. Defensive strategies showed by snakes included immobility, flight attempts, coiling, cocking, rattling, and counterattack with strikes and bites. The most common defensive behavior of the rattlesnakes was immobility. The way the opossums attacked was classified in three categories, depending on the defensive reactions presented by the snakes. On all occasions when the opossums were bitten, the injection of venom apparently did not affect the predation. The great ability in capturing and handling *Crotalus durissus* together with the apparent great tolerance to the venom shown by *Didelphis marsupialis* when preying on these snakes confirms the existent biochemical and immunological data about the resistance of opossums to crotalic venoms. In this way our data strongly reinforce the supposition that this species is an effective snake predator in nature.

Key words: Marsupialia; Behavior; *Didelphis*; Venom; *Crotalus*

INTRODUCTION

Didelphis marsupialis is a very common marsupial in Brazil, living mainly in forests

(Cerqueira, 1985). It is found in environments modified by humans, adapts well in urban areas, and is nocturnal, scansorial, and omnivorous. Its diet is composed of fruits, nectar, small vertebrates, and invertebrates (Emmons, 1990). Many reports cite opossums as natural predators of snakes including venomous species (Silva Jr., 1956; Fitch, 1960; Cordero and Nicolas,

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1986; Sazima, 1992). In addition, Brodie III (1993) observed attacks of opossums towards snake replicas in the field. At the park of Butantan Institute, São Paulo, Brazil, attacks by native opossums on the outdoor enclosure snakes have been often reported (W. Fernandes, pers. commun.).

A number of studies is available demonstrating that opossums are immune to the venom of some viperids (Domont et al., 1991). Besides the well-known immunity against *Bothrops* venom (Moussatché et al., 1979, 1990; Perales et al., 1994; Neves-Ferreira et al., 1997), resistance of *Didelphis* to the venoms of *Crotalus durissus* (Vellard, 1945; Moussatché et al., 1979, 1990) and *C. atrox* (Werner and Vick, 1977; Perez et al., 1979) has been observed. All these data were obtained from biochemical and immunological assays. Studies about the zoological implications of this resistance have never been undertaken.

Information about the predatory behavior of American marsupials is scarce. Sazima (1992) made a single observation on the behavior of *Didelphis* toward its prey; his work, however, was not specific to predation. Jared et al. (1998) made a few preliminary observations on young opossums attacking and killing young *Bothrops jararaca* in captivity. Despite the strong supposition about opossums being effective predators of viperids, experimental results demonstrating this fact do not yet exist.

On the other hand, although snakes present the most elaborate antipredator mechanisms hitherto described among reptiles, few papers are found about the defensive behavior of these animals toward their effective predators (Greene, 1973, 1988). Among venomous snakes, rattlesnakes are a differentiated group possessing a unique structure, the rattle, specialized in sonorous defensive signaling (Greene, 1988). According to Duvall et al. (1985) and Greene (1988), in nature the whole set of defensive behaviors in rattlesnakes corresponds to an increase of aggressiveness comprising

procrypsis (immobility associated with a cryptic coloration pattern), flight, body coiling, cocking (retracting of the coiled body and intimidation with strikes), rattling, head hiding, strikes and bites, and finally emptying of the anal glands. This set of behaviors or, in most cases, the combination of some of them, is sufficient to prevent the snakes from being killed by predators.

This paper describes a behavioral experiment in the laboratory where *Crotalus durissus* was offered to *Didelphis marsupialis*. It aims to understand the defensive and attack strategies of both animals. It also has the intention of comparing the obtained behavioral data with the existent biochemical and immunological information about the resistance of *Didelphis* to *Crotalus* venom. Drawing on these results, this work finally tries to make a few inferences about the predatory and defensive behaviors of these animals in nature.

MATERIALS AND METHODS

Animals

Healthy adults *D. marsupialis* (N = 12) collected from the woods of Butantan Institute and from the surroundings of São Paulo city were used. They weighed 1.5 to 2 kg.

The opossums were maintained in individual tanks measuring 0.80 × 0.70 m and 0.70 m in depth, and closed with a wire netting lid. A wooden box measuring 0.27 m in width, 0.34 m in length, and 0.20 m in height with an inclined wire netting front door was placed inside the tank for shelter. Due to its design, the box did not allow the opossum in the shelter to see the environment inside the tank. This shelter box occupied 16% of the total area of the tank and was removed daily from the tanks for cleaning, with the animal inside. After that, the tanks were lined with cardboard, and the box was replaced and reopened. Water and food (fruits and

minced meat) were supplied daily. Opening and closing of the box door were done with a hook-tipped stick.

Farmers from the State of São Paulo (Brazil) supplied the snakes to the Herpetology Laboratory of Instituto Butantan. Thirty-six healthy adult *Crotalus durissus* (total length = 60 to 90 cm) were used. They were maintained in captivity for at least 20 days without being fed before the experiments in order to guarantee that the venom glands were full of secretion.

Behavioral evaluation

The observations were conducted during a two-year period. Before initiating the experiments each animal was kept in captivity at least for a month for acclimation to the new environment. Each opossum was tested with one snake every 15 days for three times per opossum. Before each observation the opossums were fasted for two days. The experiments were conducted in darkness at night, the period when the animals are active. The tank was lighted inside with a 15 W lamp and closed with a glass plate instead of the netting lid. With this system the opossums were kept acoustically and visually isolated from the outside environment. At the same time, the inclined door of the box made it possible for the observers to see the opossum inside the box from top, through the glass plate. The animal was kept in this condition for 1 hr before the snake was offered. The system allowed the option for the opossum of attacking the snake or remaining inside the box. Observations were started as soon as a snake was gently placed into the tank. Twenty-five out of a total of 36 experiments were recorded on video tape and photographed. In the experiments where predation did not occur the total time of observation was 120 minutes. Five control experiments were conducted placing a snake alone into the tank and recording its behavior on video tape for 1 hr.

A binomial test was used to statistically

test the preference for tail attack. The null hypothesis was $p = 0.5$.

RESULTS

The opossum, even at night, constantly remained inside the shelter box. When food was placed in the tank, the opossum immediately started to smell it with visible movements of its snout and, in most cases, came out of the box to feed on it. Sometimes, however, after smelling the food for a few minutes it remained inside the box.

Two distinct phases of behavioral interactions between the opossum and the rattlesnake were characterized after the snake was placed in the tank: 1) behaviors before the attack, including the observations before the opossum left the box and 2) the attack itself, that was defined here as the set of behaviors observed from the moment the opossum came out of the box and approached the snake until effective predation occurred.

Before the attack, the opossum, inside the box, immediately smelled the snake for a few seconds to 2 min. During this time different reactions of the snake could be observed: 1) calm movements around the tank, 2) a quiet coiled posture, 3) immobility (freezing), 4) flight, 5) threatening coiled posture (cocking) or 6) rattling. The opossum, after smelling the snake, sometimes remained in the box, not leaving to catch it. In four experiments, when the opossum inside the box spent more time smelling the snake, rattling was observed; in all these occasions the opossum chose to remain in the box, not attacking the snake.

When the opossum attacked, it came out of the box, approached the snake quickly (Fig. 1A), and captured it (Fig. 1B). On three occasions, the attack was so quick that the snake had no time to react. Most times, however, the snake showed some type of reaction.

Three types of behavioral interactions were recognized during the attacks. Figure

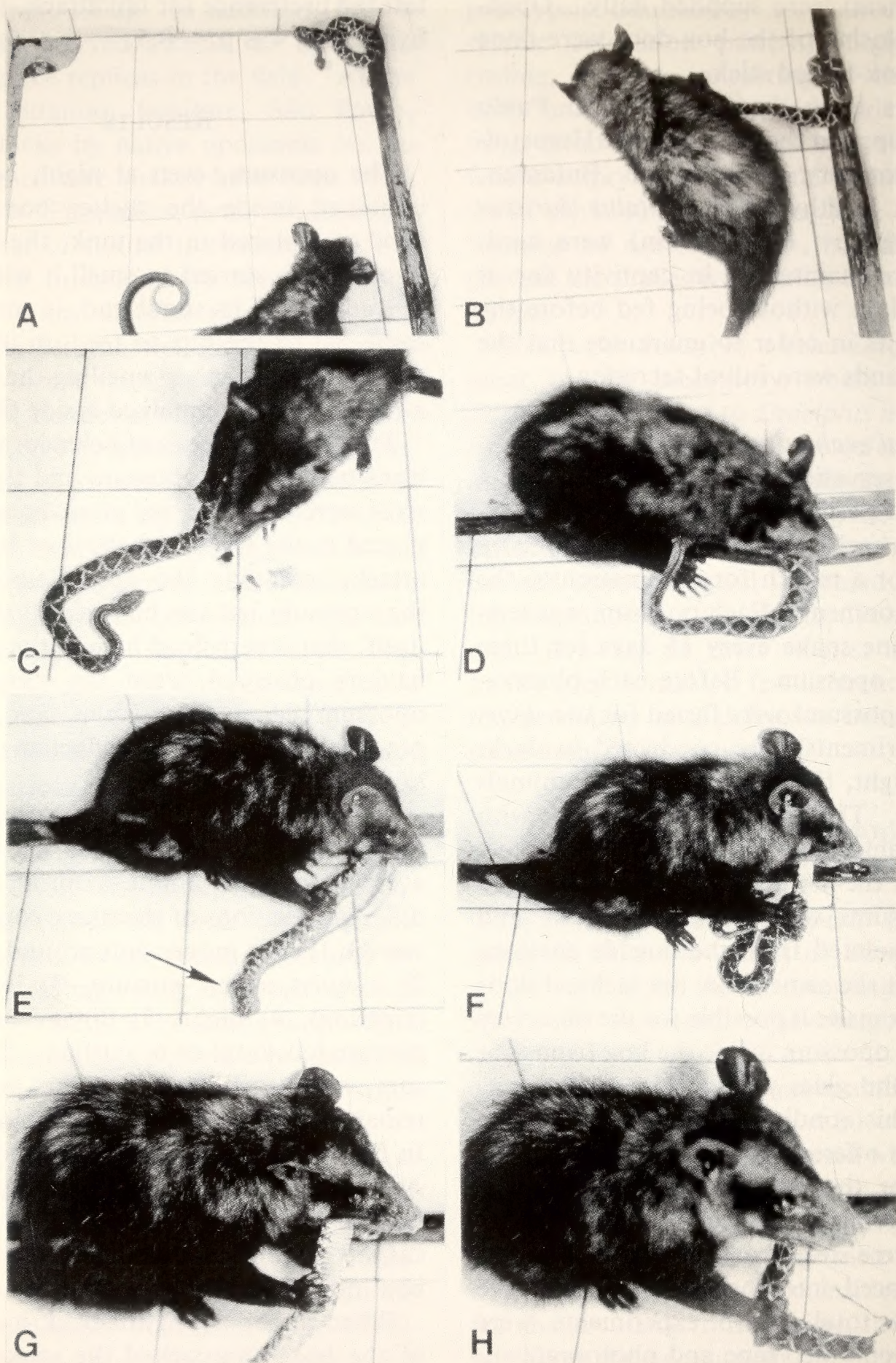


FIG. 1. One of the possible sequences of predatory behaviors of the opossum on the rattlesnake. A: The opossum directly approaches the snake, which remains immobile. B: The opossum captures the snake by the tail. The snake remains passive, flicking the tongue. C: The opossum continues eating the snake from the tail. The snake still flicks its tongue. D: The snake suddenly bites the opossum. E: The opossum reacts, kills the snake by chewing its head (arrow) and bites the rest of the body. F to H: The opossum continues eating the snake from the anterior end. While eating, it remains in the same position (F, G) until it finishes the whole snake (H).

2 summarizes the feeding tactics of the opossums, which depended on the reactions exhibited by the snakes after the initial approach:

- (1) The snakes showed an erratic behavior, moving the body by chance in a disoriented manner. In this case, the opossum immobilized them with successive bites along the whole body. Then it consumed them from either the tail or the head.
- (2) The snakes were immobile. In this case the opossum consumed them alive, preferentially from the tail (Fig. 1C). Usually the snakes stayed immo-

bile throughout predation. Sometimes, however, the snakes counterattacked with part of their bodies already eaten.

- (3) The snakes counterattacked with strikes and bites either just before being captured or having already part of its body eaten from the tail (Fig. 1D). In both cases the opossum killed them by chewing the head, and then immediately consumed them beginning from one of the extremities (Fig. 1E).

Besides these three types of interactions, a few encounters were observed where the opossum and the snake faced each other

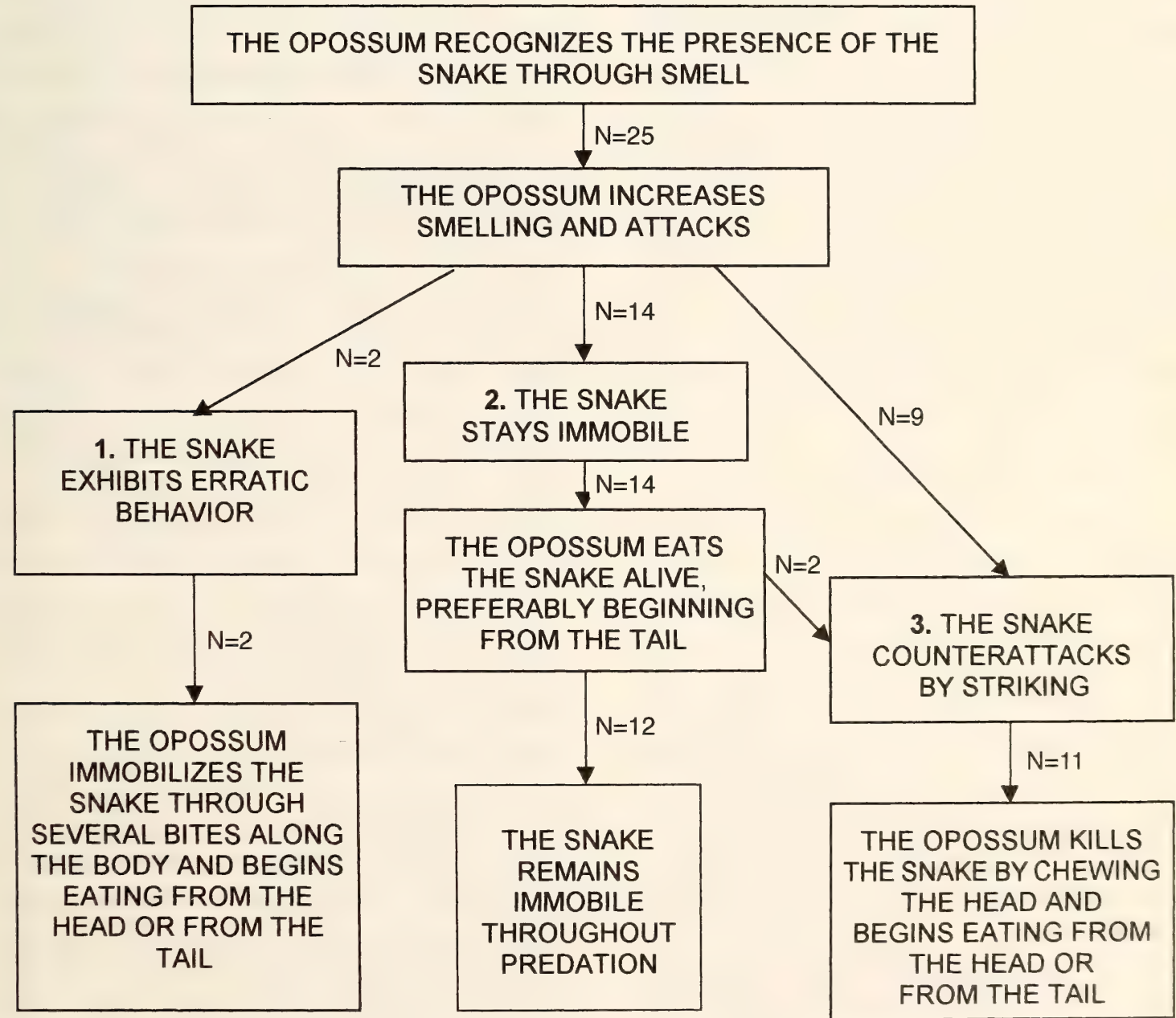


FIG. 2. Scheme of attack and defense strategies in the encounters of opossums and snakes. Three different types of reaction are observed in the rattlesnakes after the first attack by the opossum. N = number of filmed events.

without moving for a long time. The two animals remained in this position until the snake slowly moved backwards.

During predation, the prey handling behavior was quite regular: the opossum remained in a sitting position while holding the snake with one of the forelimbs (Figs. 1F, 1G). One of the ends of the snake was introduced into the mouth laterally and was chewed with the lateral teeth (Figs. 1F, 1G). The other forelimb supported the animal (Figs. 1F, 1G). The forefeet were used alternately but for short periods of time were used together to tear apart the harder parts of the snake. Generally the whole snake was eaten uninterruptedly (Fig. 1H) with an average duration of 12 min.

The opossums killed and ate approximately 80% of the offered rattlesnakes. Taking into account the filmed captures where the attack was directed to the tail or to the head ($N = 22$), the opossums seemed to direct the attack significantly to the tail ($N = 16$; $p < 0.05$, $df = 1$). In three filmed captures the opossums directed the attack to the middle of the body.

During all experiments no observable effects of envenomation were recognized.

DISCUSSION

The way the opossums behaved while eating the snakes was very regular. The opossums were always in the same posture when observed. They used only one forelimb at a time to hold the snake and introduce it into the mouth. Ivanco et al. (1996) also verified the use of a single limb in another opossum species, *Monodelphis domestica*, when feeding or preying, and suggested that this behavior is fixed and species-typical.

During the time preceding the effective predation, when the encounter between opossum and rattlesnake took place, great behavioral variations were noted in both animals.

Before the attack by the opossum, the differences in reaction presented by the rat-

tlesnakes indicated that some times they were not able to notice the opossum, moving calmly around the tank or remaining quietly coiled. Most times, however, the rattlesnakes demonstrated by their behavior that they could recognize the opossum as a predator; in these cases some of the typical behaviors of the defensive escalation of the rattlesnakes were observed, such as immobility, coiling, cocking, and rattling. Among these defensive behaviors, the strategy of immobility in nature can be very valuable when associated with a cryptic coloration pattern and may constitute an efficient defense used by these animals against predators (Greene et al., 1978; Herzog and Drummond, 1984; Cloudsley-Thompson, 1994). Rattling many times occurred despite the snake not being able to see the opossum inside the box. Since it was not observed in any of the control experiments, rattling indicates that most times the snakes seemed to identify somehow the danger, possibly by chemical signs, as proposed by Weldon et al. (1992).

On a significant number of occasions the attack of the opossum was directed to the tail. That was observed more frequently when the rattlesnakes remained immobile, a strategy that at first view is difficult to interpret. On the other hand, during the approach, it was observed on a few occasions that the opossum, when noticing the snake coiling and preparing to strike, rapidly killed it by attacking and chewing its head. The same happened in the few cases when the rattlesnakes were able to bite the opossum even after being captured by the tail. In other cases, when the snake reacted to the attack by exhibiting erratic behavior, it was immobilized through bites along the whole body. The analysis of these different situations observed during the attack indicated that any type of active reaction presented by the snake caused an immediate fatal attack by the opossum. Although in our experimental conditions attacks directed to the tail did not prevent the snake from

being killed by the opossum, in nature they may confer an advantage to the snake, that by remaining immobile has a chance of escaping without being severely injured, as has been already observed for lizards by Greene et al. (1978). These data seem to be in accordance with Herzog and Burghardt (1974), who affirmed that for many predators, prey movement is a critical factor in mediating attack.

In contrast to our results indicating some preference of the opossums for capturing the snakes by the tail, Sazima (1992) reported that *D. marsupialis* when attacking *Bothrops jararaca* usually goes first to the head or neck region. In our experiments with *Crotalus durissus*, in many cases, *Didelphis* grasped the tail first, giving the snakes a chance to bite. This observation, at first view, seems to be contradictory since the predatory behavior of ophiophagous animals (mammals or birds) usually consists in attacking the head or the region just behind the head (Kaufmann and Kaufmann, 1965; Perez et al., 1978). However, ophiophagous animals such as *Conepatus* sp. and *Galictis* sp. have been observed attacking snakes at the tail (Ribeiro, 1940; Jackson, 1979).

It is possible that in our study the apparent preference of *D. marsupialis* for attacking the tail of *C. durissus* may be caused by an attraction of the opossums to cloacal odors of the rattlesnakes that can misdirect the attack. In nature, such attraction of the predator to the tail, which is a more disposable portion of the body, could help the prey to escape or counterattack (Greene, 1988; Alcock, 1993).

Two species of opossums of the genus *Didelphis* occur in Brazil: *D. albiventris* and *D. marsupialis* (Cerqueira, 1985). The former lives in open fields such as "cerrado" and "caatinga" and the latter is distributed in forests (Cerqueira, 1985; Emmons, 1990). On the other hand, *Crotalus durissus* is a species typical of open fields while *Bothrops jararaca* is distributed in forests

(Sazima, 1992; Campbell and Lamar, 1989). In this way, one would expect the opossums to have resistance only to snake venoms from the same habitat. In fact, Mous-satché et al. (1990) have demonstrated that *D. marsupialis* remains unharmed by *B. jararaca* venom. In addition, they mentioned that this marsupial has partial resistance to *Crotalus durissus* venom. On the basis of this information, our experiments aimed at comparing behavioral results with the biochemical data of Mous-satché et al. (1990). Although *D. marsupialis* and *C. durissus* are not sympatric in nature, we observed that, at least in captivity, predation was effective. The injection of venom by snakebites apparently did not affect the predation as has already been observed for other predators, including mammals and birds (Duvall et al., 1985).

In spite of the limitations imposed by a behavioral experiment conducted in captivity, the present observations strongly suggest that *D. marsupialis* is an effective snake predator in nature. This supposition is mainly based on the great interest and ability shown by *D. marsupialis* in capturing *Crotalus durissus*, which were comparable to the interest they showed when presented with different types of food. This idea is also reinforced by the great tolerance these animals showed to the snake venom.

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***Aphaniotis nasuta* (de Jong, 1930), a Junior Synonym of *A. ornata* (Van Lidth de Jeude, 1893) (Squamata: Agamidae)**

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Abstract: Morphological comparisons of available types of the two snout-ornamented agamids from northern Borneo, *Aphaniotis ornata* and *A. nasuta*, failed to show any substantial differences. Careful examination of original descriptions of these nominate taxa also yielded no discriminant characters. Thus, although the holotype of *A. nasuta* was not detected in our survey of various museum collections, we are sure that it is appropriate to synonymize this nominate species with *A. ornata*.

Key words: *Aphaniotis nasuta*; *Aphaniotis ornata*; Agamidae; Synonymy; Borneo

INTRODUCTION

Van Lidth de Jeude (1893) described *Japalura ornata* on the basis of a female agamid with long limbs and a rostral appendage from near Sandakan Bay, North Borneo (i.e., Sabah, Malaysia). He assigned this species to *Japalura* chiefly because of the presence of an oblique fold in front of the shoulder. Later, de Jong (1930) described another long-limbed, snout-ornamented agamid species, *Japalura nasuta*, on the basis of six specimens also from North Borneo. He, however, did not compare this species with *J. ornata*, nor

even mention the latter.

In his doctoral dissertation, Moody (1980) made drastic changes in the classification of the family Agamidae, which involved translocations of *ornata* and *nasuta* from *Japalura* to another genus, *Aphaniotis*. He did not mention any concrete reason for such rearrangements, but it is almost certain that these and other changes proposed in his taxonomic list (given as Appendix A) reflect his morphological redefinitions of genera resulting from phylogenetic analyses of the whole family (Moody, 1980).

Despite the ambiguity regarding the validity of *nasuta* in the presence of *ornata* (see above), almost no one subsequent to de Jong (1930) has addressed this taxonomic problem, and only Manthey and Grossmann (1997) pointed out the necessity for

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the clarification of their differences. Thus, both *ornata* and *nasuta* have been literally regarded as valid species of *Japalura* (e.g., Wermuth, 1967), or of *Aphaniotis* (e.g., Welch et al., 1990; Welch, 1994).

Recently we examined type specimens of both of these species. The Results of comparisons strongly suggest that these nominate species are actually conspecific.

MATERIALS AND METHODS

The holotype of *A. ornata* (RMNH 4344:

Fig. 1a) and a paratype of *A. nasuta* (SMF 78702: Fig. 1b), both adult females, were examined. Definitions of quantitative characters follow Ota (1991). Institutional acronyms are those suggested by Leviton et al. (1985).

RESULTS AND DISCUSSION

Table 1 compares 15 quantitative characters of the types of *A. ornata* and *A. nasuta*. These specimens greatly resembled each other in most characters examined. The

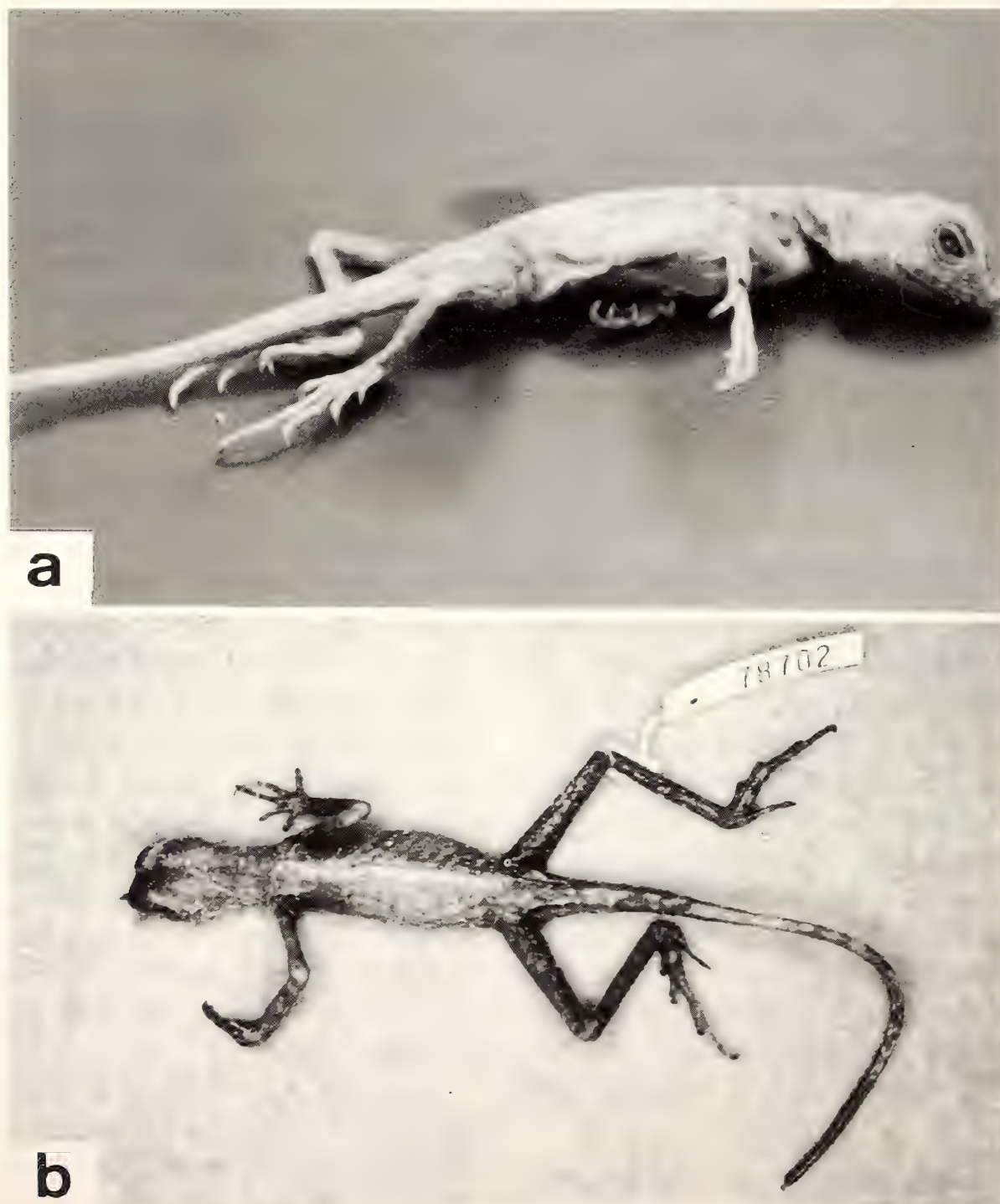


FIG. 1. Holotype of *Aphaniotis ornata* (RMNH 4344, SVL = 54.2 mm) (a), and a paratype of *A. nasuta* (SMF 78702, SVL = 53.5 mm) (b).

TABLE 1. Comparisons of meristic and morphometric characters (in mm) between holotype of *Aphaniotis ornata* (RMNH 4344) and a paratype of *A. nasuta* (SMF 78702). Abbreviations are as follows. SL: supralabials; IL: infralabials; IO: interorbital scales; MSR: scale rows around midbody; FIVS: finger IV subdigital scales; TIVS: toe IV subdigital scales; SVL: snout-vent length; HL: head length; HW: head width; SFL: snout-forelimb length; AGL: axilla-groin length; FLL: forelimb length; HLL: hind-limb length; FIVL: finger IV length; TIVL: toe IV length.

Character	<i>A. ornata</i>	<i>A. nasuta</i>
SL	8	8
IL	7	7
IO	19	22
MSR	80	77
FIVS	18	19
TIVS	21	24
SVL	54.2	53.5
HL (ratio to SVL)	14.0 (25.8%)	14.8 (27.7%)
HW (ratio to SVL)	9.9 (18.3%)	9.7 (18.1%)
SFL (ratio to SVL)	22.4 (41.3%)	21.1 (39.4%)
AGL (ratio to SVL)	25.5 (47.0%)	24.4 (45.6%)
FLL (ratio to SVL)	29.0 (53.5%)	31.0 (57.9%)
HLL (ratio to SVL)	51.1 (94.3%)	54.9 (102.6%)
FIVL (ratio to SVL)	7.6 (14.0%)	7.8 (14.6%)
TIVL (ratio to SVL)	10.6 (19.6%)	11.5 (21.5%)

possible greatest differences lay in the relative fore- (FLL) and hindlimb lengths (HLL) that were somewhat greater in the paratype of *A. nasuta* (57.9% and 102.6% of the snout-vent length [SVL], respectively) than in the holotype of *A. ornata* (53.5% and 94.3%, respectively). Even so, such differences, corresponding to 4.4% of SVL in FLL and 8.3% of SVL in HLL, are well within the extent of variations in corresponding characters among conspecific females from limited geographical ranges reported for other arboreal agamids (e.g., 5.9% of SVL in FLL and 9.3% of SVL in HLL for *Calotes cristatellus* from Sabah [Ota and Hikida, 1991], and 8.5% of SVL in FLL and 12.7% of SVL in HLL for *Japalura swinhonis* from Taiwan [Ota, 1991]).

Between the two specimens, there were also no differences evident in qualitative characters, such as the shape of the rostral appendage and overall coloration. Careful

comparisons of original descriptions of *Aphaniotes ornata* and *A. nasuta* (e.g., Van Lidth de Jeude [1893] and de Jong [1930], both as *Japalura*: see above) also failed to reveal any substantial differences between these nominate species.

According to de Jong (1930), the type series of *A. nasuta* consisted of two males (including the holotype) and four females, all deposited in the Buitenzorg Museum, Java. This museum was largely succeeded by Museum Zoologicum Bogoriense (MZB) after World War II. Indeed, the SMF specimen examined by us was labeled as “Formerly in Mus. Bogor.” However, despite our intensive survey of various museum collections including that of MZB, we did not find the holotype or any of the remaining paratypes of *A. nasuta*.

We consider it to be best at present to synonymize *A. nasuta* with *A. ornata*, because comparisons both of available types and of original descriptions strongly suggest

their identity as mentioned above. Further efforts should be made to detect other types, especially the holotype, of *A. nasuta* to verify this account.

ACKNOWLEDGMENTS

We thank M. S. Hoogmoed (RMNH) and G. Köhler (SMF) for the arrangement to examine specimens under their care. H. Ota also thanks the staff of MZB for responding to his query regarding the types of *Aphaniotis nasuta*. This research was supported by a Grant-in-Aid from the Japan Ministry of Education, Science, Sports and Culture (Oversea Research No. 10041166) through the courtesy of M. Matsui.

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Conspecific and Heterospecific Pair-formation in *Rana porosa brevipoda* and *Rana nigromaculata*, with Reference to Asymmetric Hybridization

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Abstract: Patterns of conspecific and heterospecific pair-formation in *Rana porosa brevipoda* and *Rana nigromaculata* were investigated in the field. In *R. porosa brevipoda*, most of the conspecific pairs were formed by female initiation. Size-assortative mating was observed in *R. porosa brevipoda*. On the other hand, most of the conspecific pairs of *R. nigromaculata* were formed by forced clasping by males. There was no significant correlation between the male and female sizes in amplexant pairs of *R. nigromaculata*. Of a total of 12 heterospecific pairs observed, 11 were pairs between male *R. nigromaculata* and female *R. porosa brevipoda*. The proximate factors of the asymmetry in heterospecific pairing are discussed with relation to the differences in the pairing patterns, body size, and the body shape between the two species. Possible impacts of the asymmetric hybridization on the two species are also discussed.

Key words: *Rana porosa brevipoda*; *Rana nigromaculata*; Pair-formation; Heterospecific pairing; Hybridization

INTRODUCTION

The Japanese pond frogs *Rana porosa brevipoda* and *Rana nigromaculata* are closely related phylogenetically and have similar ecological requirements (Maeda and Matsui, 1989). Postmating reproductive isolating mechanisms between these two species are quite incomplete. Although male hybrids are sterile, females hybrids are mostly fertile (see Maeda and Matsui, 1989;

Matsui, 1996 for review). Currently, natural hybridization between these two species has been reported at numerous localities of their sympatric ranges (Nishioka et al., 1981, 1992). However, there is no study on the ethological and ecological factors causing natural hybridization between these two species.

In the northern part of the Ina Basin, Nagano Prefecture (central Japan), an isolated population of *R. porosa brevipoda* is distributed sympatrically with *R. nigromaculata*, which has a wide range of distribution (Nishioka et al., 1981; Shimoyama,

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1986a). In this region, frogs with intermediate external characters, which are considered to be natural hybrids, are also found (Shimoyama, 1986a, 1996). Actually, Nishioka et al. (1981, 1992) showed evidence of introgressive hybridization between the two species in this region based on the electrophoretic analyses of allozymes and blood proteins.

In the studies of comparative reproductive ecology and interspecific relationships of the two pond frogs, Shimoyama (1996) clarified the absence of premating reproductive isolation between the two species in the northern Ina Basin. Shimoyama (1996) pointed out the absence of distinct segregation in diel, seasonal, and spatial patterns of breeding activity between the two species, which results in the formation of mixed-species choruses comprising males of both species. Such a mixed-species chorusing is caused by the misidentification of heterospecifics as conspecifics by male *R. nigromaculata* (Shimoyama, 1999). Shimoyama (1999) further documented similarities of vocal repertoires, advertisement call structures, and male social behavior between the two species. In addition, Shimoyama (1999) demonstrated the occurrence of heterospecific pairing and spawning within the mixed-species choruses.

In the present paper, I first describe patterns of conspecific and heterospecific pair-formation of the two species, with relation to the asymmetry in the heterospecific pairs and hybridization. I then discuss the possible proximate factors and influences of the asymmetric hybridization.

METHODS

Study area

This study was done in the breeding seasons of 1990, 1992, 1993, and 1995–1997 in Tatsuno (35°56' N, 137°58' E, 720 m above sea level), Nagano Prefecture, central Japan. A detailed description of the study site is given in Shimoyama (1996).

Observations

Field survey was made on 47 days in May–July 1990, five days in May 1992, six days in May 1993, eight days in May–July 1995, 18 days in May–June 1996, and six days in May–June 1997. Details of the general methods are described in Shimoyama (1996, 1999).

Observations on male behavior within the mixed-species choruses were made usually at 0500–0700 hr, when males and females of both species are most active (Shimoyama, 1996). When breeding activities were still very intense after 0800 hr, observations were continued until 1000 hr. The sum of behavioral observations was 265 hours. On every visit, I selected one or two mixed-species chorus(es) for observation of male and/or female behavior from nearby (usually < 5 m) banks. Binoculars ($\times 7$) were used to ascertain individual identification. When gravid females were found around or within the choruses, I started the focal animal sampling (Martin and Bateson, 1986) for the females until they finished spawning.

After spawning, the number of eggs within each clutch was counted for the clutches of *R. porosa brevipoda* to determine whether it was a first or second clutch (see Serizawa, 1983, Shimoyama, 1986b). I could judge the clutches to be first or second based on the relationship between female snout-vent length (hereafter SVL) and the number of eggs within the clutch (see below and Serizawa et al., 1990).

When heterospecific spawnings were observed, I monitored the egg masses for several days to determine whether they hatched normally or not. If at least a part of the eggs hatched, I considered that hybridization had occurred.

RESULTS AND DISCUSSION

Breeding of R. porosa brevipoda

Size distribution of mature *R. porosa brevipoda* is shown in Fig. 1. Data from

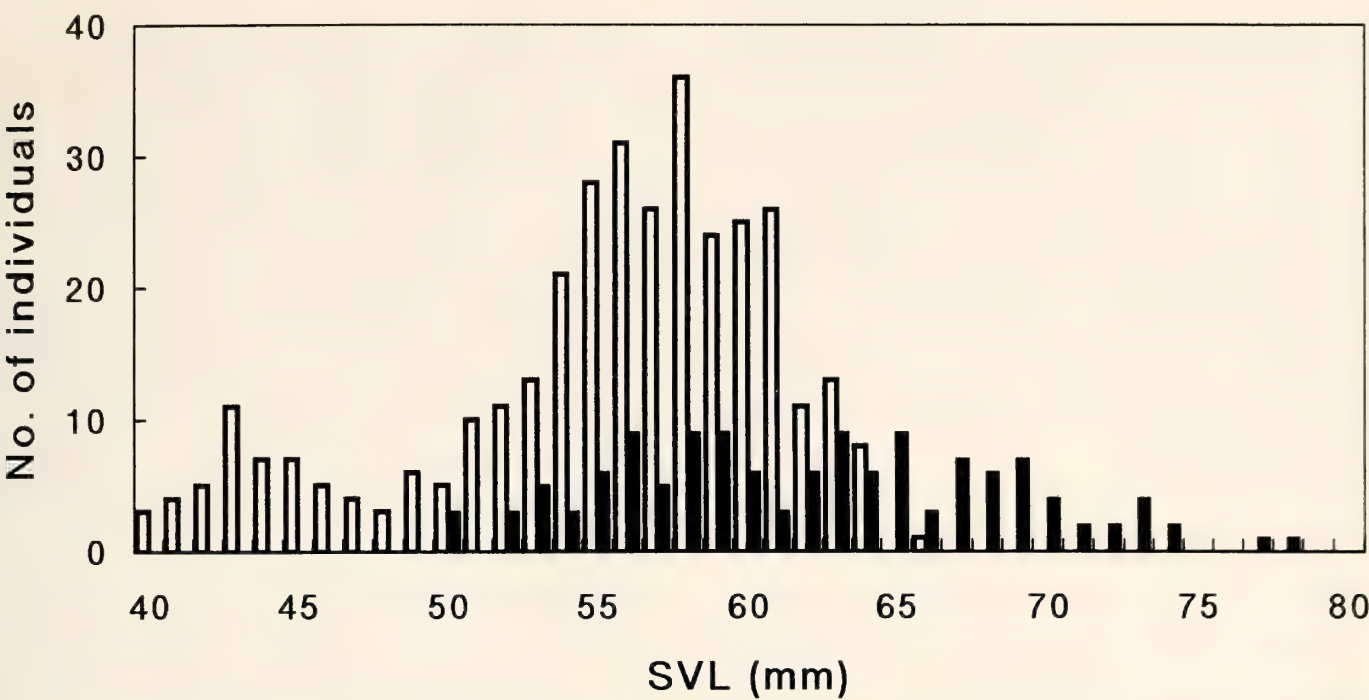


FIG. 1. Size distribution of mature individuals of *Rana porosa brevipoda*. Open and closed histograms show males and females, respectively.

the seven seasons were pooled. Histograms of the mature males (hereafter males) show two distinct size groups. One is composed of smaller individuals with SVL of 40–50 mm, and the other is composed of larger

individuals with SVL of more than 50 mm. The former group is estimated to be composed of 1-yr-olds, and the latter to be composed of older animals (see Inoue, 1979; Serizawa, 1983; Shimoyama, 1989).

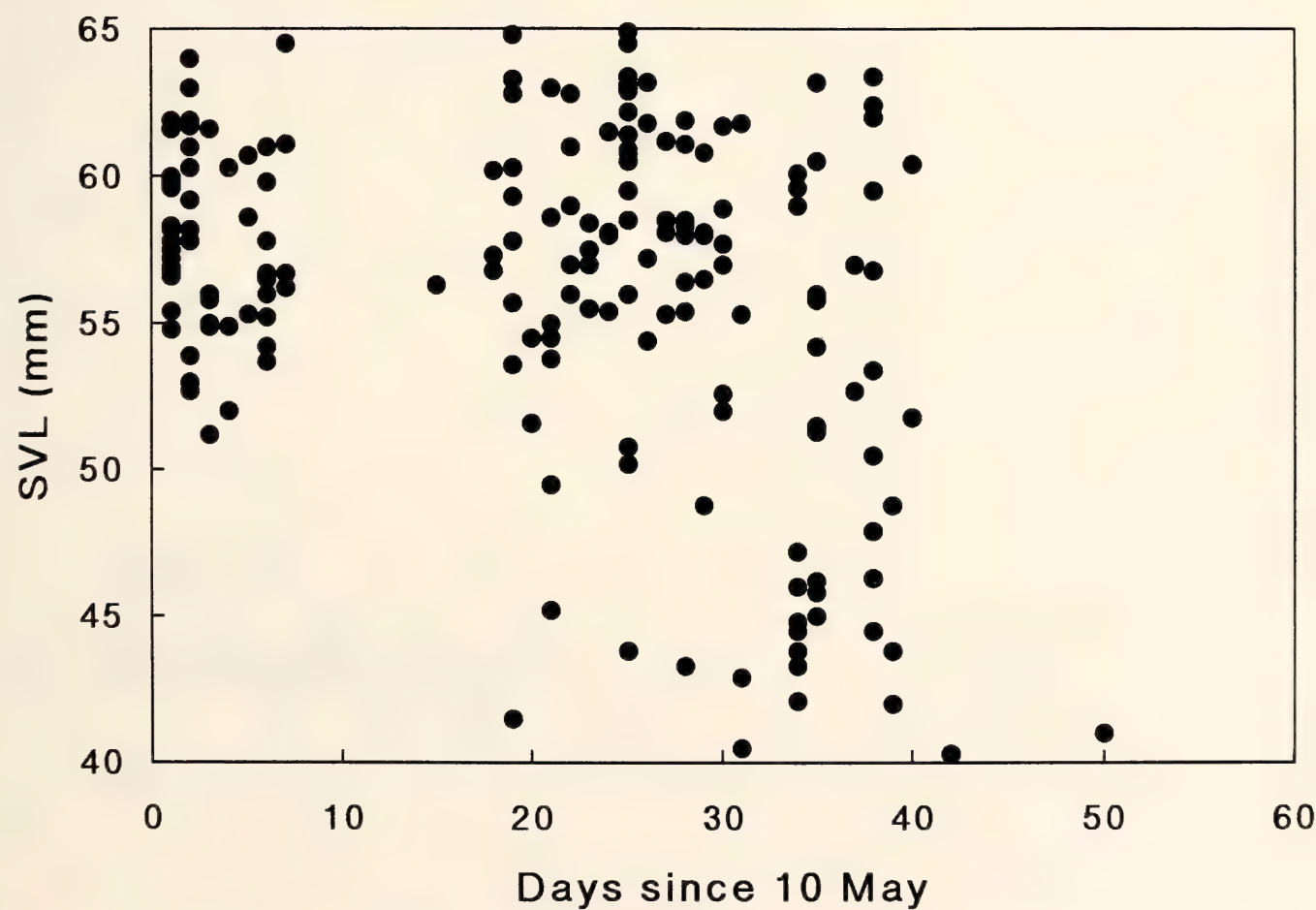


FIG. 2. Relationship between body size and the date males of *R. porosa brevipoda* were first found calling in 1990.

On the other hand, mature females (hereafter females) were not divided into distinct size groups. Females were estimated to be older than 1 year (Shimoyama, 1986b, 1989). Mean SVL of male and female *R. porosa brevipoda* was 55.7 mm (SE=0.31, N=344) and 62.5 mm (SE=0.55, N=130), respectively. Females were significantly larger than males (Mann-Whitney U-test, $z=9.03$, $p=0.0001$).

Figure 2 shows the relationship between the date of first discovery of calling and the male SVL. There was a significant tendency for smaller males to begin calling later in the season than larger males ($r=-0.332$, $N=179$, $p=0.0001$). As shown in Fig. 3, there was also a significant trend for larger males to call on more nights than smaller males ($r=0.364$, $N=179$, $p=0.0001$).

On the basis of the relationship between female SVL and number of eggs within the clutch, I could judge whether each clutch

was a first or second one (Fig. 4; see also Serizawa et al., 1990). The average number of eggs within the estimated first clutches and the second ones were 1813.0 (SE=61.42, N=28) and 556.8 (SE=57.56, N=13), respectively. Figure 5 shows the relationship between the date of oviposition and the female SVL. There was a trend for larger females to deposit first clutches earlier in the season and deposit second clutches approximately a month later. But deposition of the second clutch was not observed in females of less than 63 mm SVL.

During the course of the study, I found 62 conspecific pairs of *R. porosa brevipoda*. These pairs included unmarked individuals, whose size was not known. I observed the sequence of the pair-formation for 36 cases. As reported in Shimoyama (1993b), two major patterns of the pair-formation were found. One was female initiation, and the other was forced clasping by males. Of the

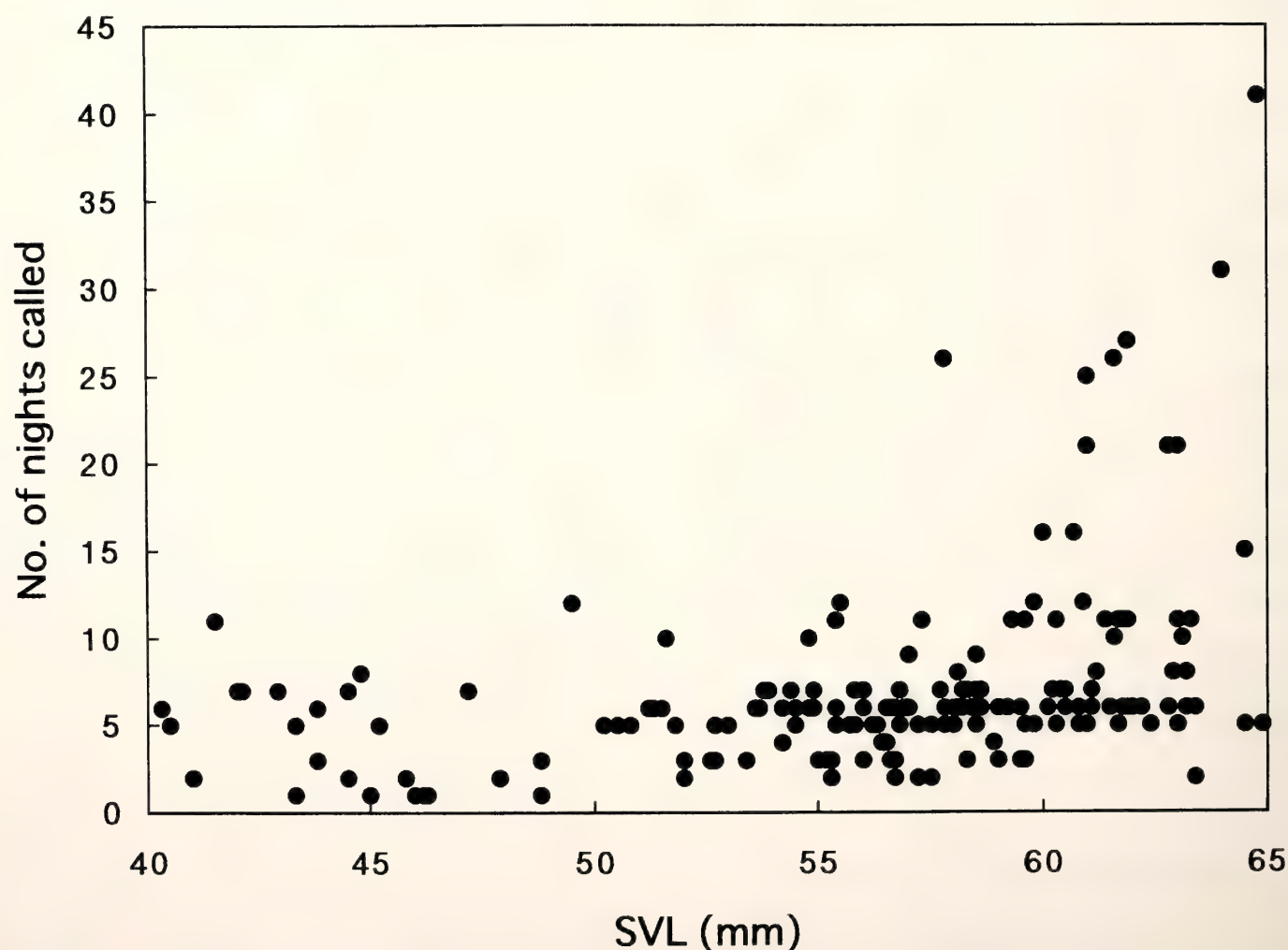


FIG. 3. Relationship between body size and the number of nights of calling in males of *R. porosa brevipoda* in 1990.

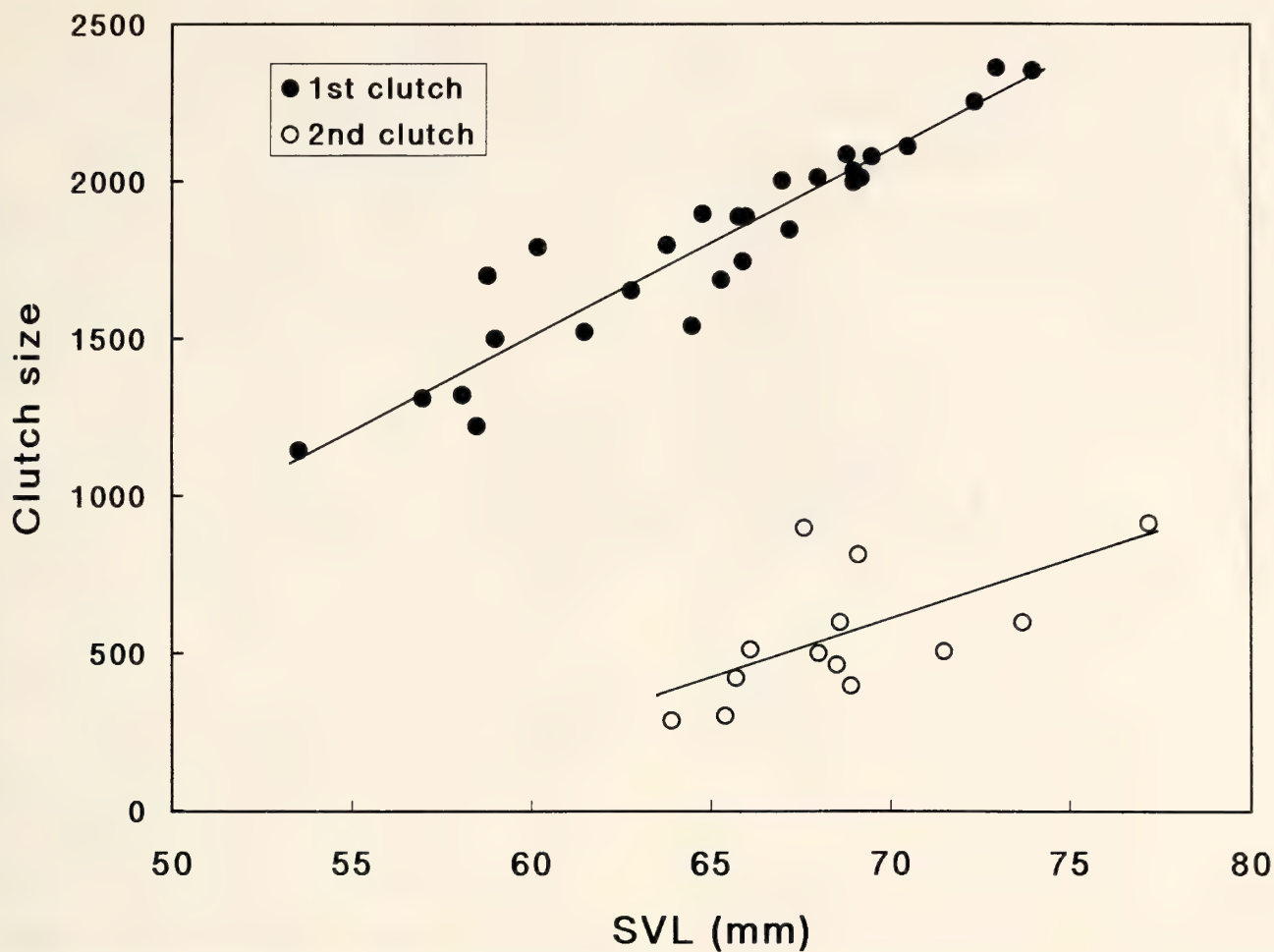


FIG. 4. Relationship between female size and the number of eggs per clutch in *R. porosa brevipoda*. Data from the seven seasons were pooled. Regression equation for the estimated first clutch is: $Y=58.5X-1996.9$ ($r=0.941$, $N=28$, $p=0.0001$), and that for the estimated second clutch is: $Y=35.4X-1880.7$ ($r=0.617$, $N=13$, $p=0.0247$).

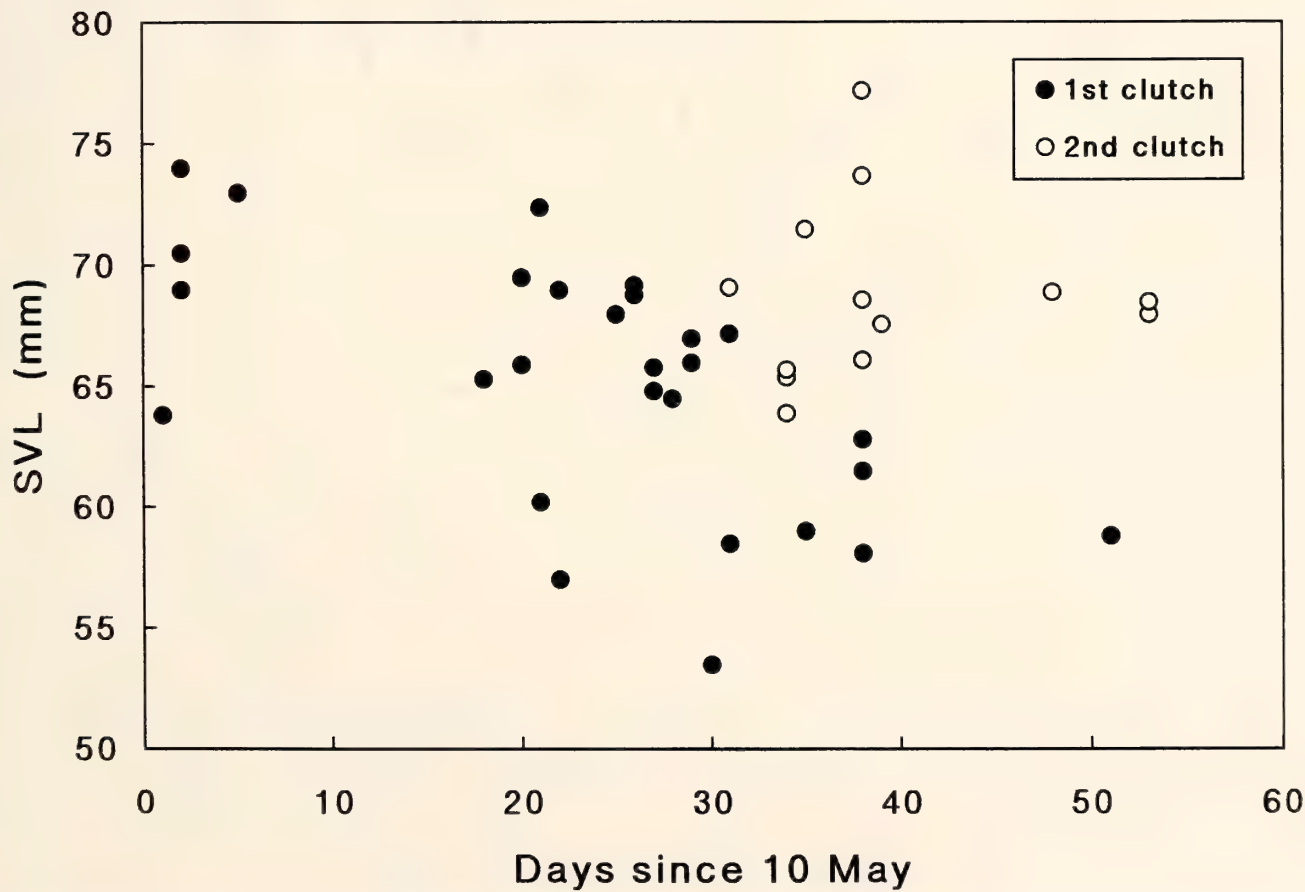


FIG. 5. Relationship between the date of oviposition and the size of female *R. porosa brevipoda* on 1990. First clutch: $r=-0.592$, $N=28$, $p=0.0009$; second clutch: $r=0.06$, $N=13$, $p>0.8$.

36 cases of the pairing sequence, 30 (83.3%) were initiated by the female. In all of the 30 cases, a gravid female visited only one calling male and mated with the first male she visited. Gravid females seemed to approach males which initiated bouts of calling more frequently. The remaining six cases of the pairing sequence were initiated by forced clasping by males: four and two females were clasped by calling males and satellite males, respectively (see Shimoyama, 1993b).

Figure 6 shows the relationship between the SVL of females and that of males in the conspecific pairs of *R. porosa brevipoda*. There was a significant positive correlation between the male and female sizes ($r=0.534$, $N=61$, $p=0.0001$). This phenomenon, which is called "size-assortative mating", has been considered to be evidence of female mate choice. However, Arak (1983) pointed out that size-assortative mating

alone cannot be regarded as evidence of mate choice by females, but that male-male competition could incidentally make a pattern of size-assortative mating. In the present study, I did not obtain adequate data to further discuss the proximate and ultimate factors of the size-assortative mating observed in *R. porosa brevipoda*.

Breeding of *R. nigromaculata*

Figure 7 shows the size distribution of mature *R. nigromaculata*. SVL of males ranged from 49.0 to 80.8 mm with a mean of 66.0 mm ($SE=0.39$, $N=172$), and that of females from 60.0 to 83.0 mm with a mean of 70.9 mm ($SE=0.67$, $N=62$). Males were estimated to be more than 1 year of age, and females to be more than 2 years of age (Shimoyama, 1989). Distinct size groups were not found in either sex. Females were significantly larger than males (Mann-Whitney U-test, $z=5.86$, $p=$

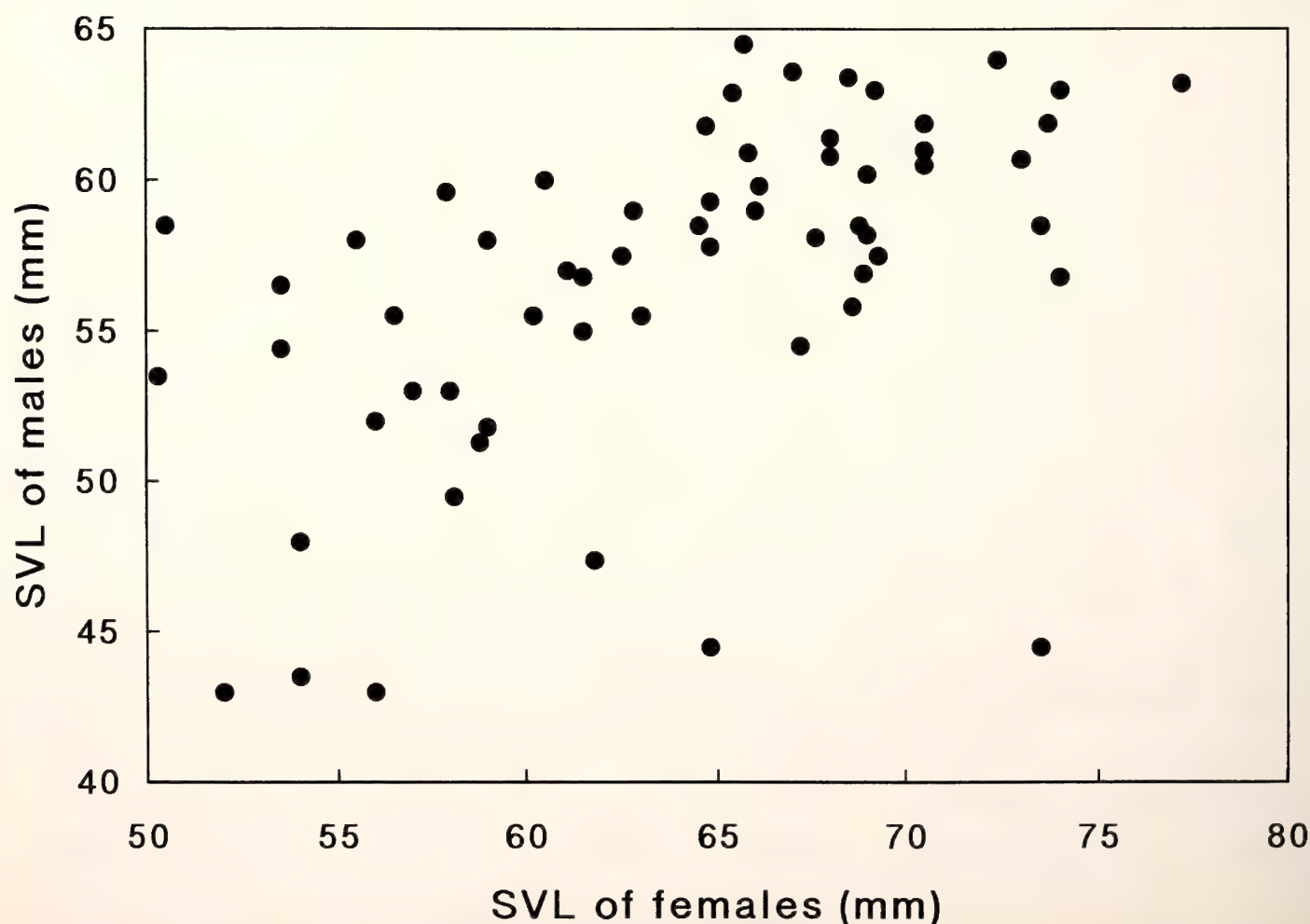


FIG. 6. Relationship between the male and female sizes in amplexant pairs of *R. porosa brevipoda*. Data from seven seasons were pooled.

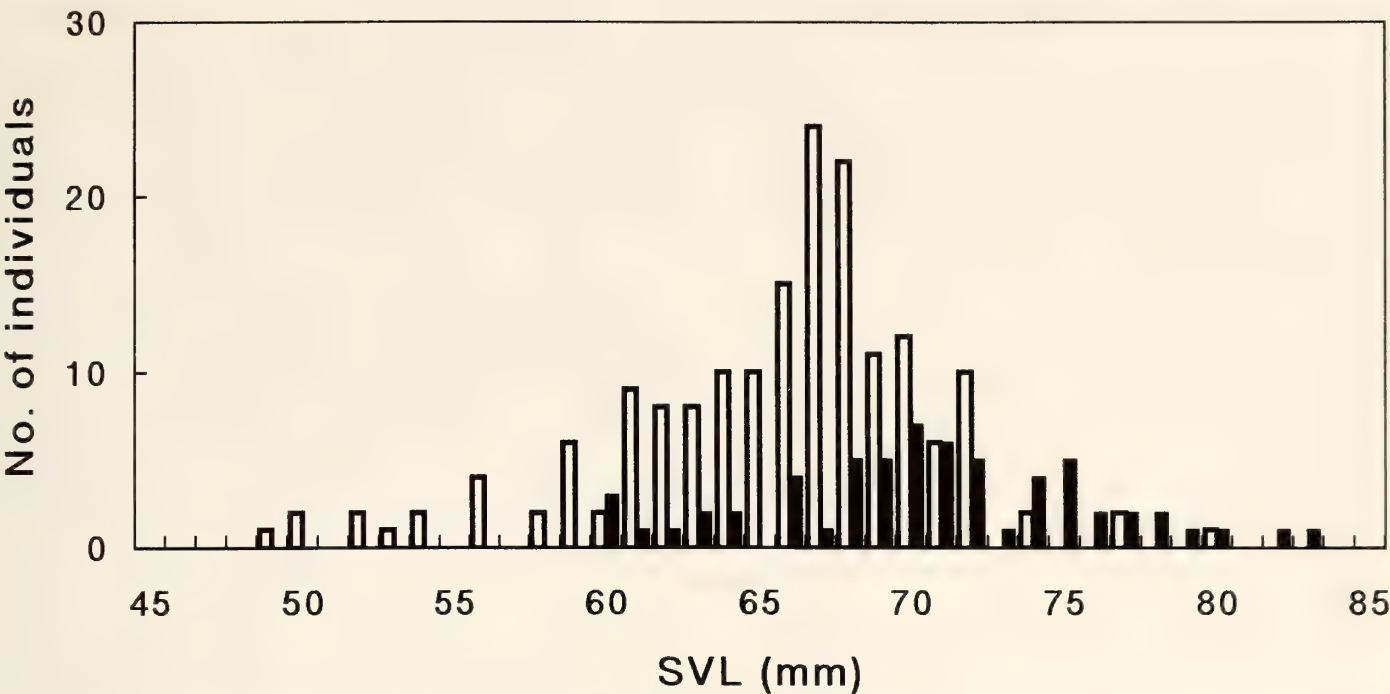


FIG. 7. Size distribution of mature individuals of *Rana nigromaculata*. Open and closed histograms show males and females, respectively.

0.0001).
There was no trend between the date of the first discovery of calling and male size in *R. nigromaculata* ($r=0.048$, $N=84$, $p>0.6$; Fig. 8). But there was a significant tenden-

cy for larger males to call on more nights than smaller males ($r=0.598$, $N=84$, $p=0.0001$; Fig. 9). Figure 10 shows the relationship between the date of oviposition and female SVL in 1990. There was a sig-

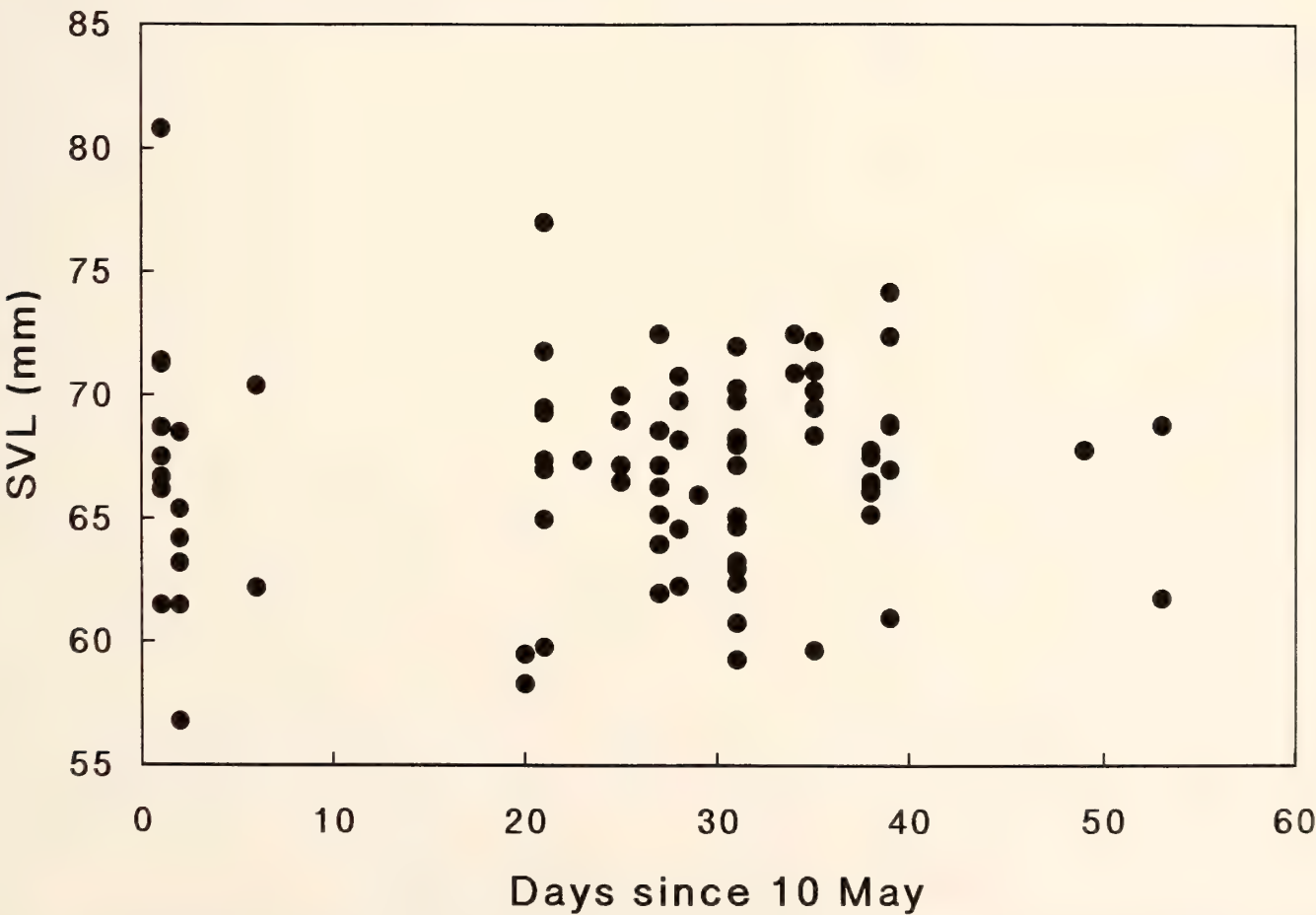


FIG. 8. Relationship between body size and the date males of *R. nigromaculata* were first found calling in 1990.

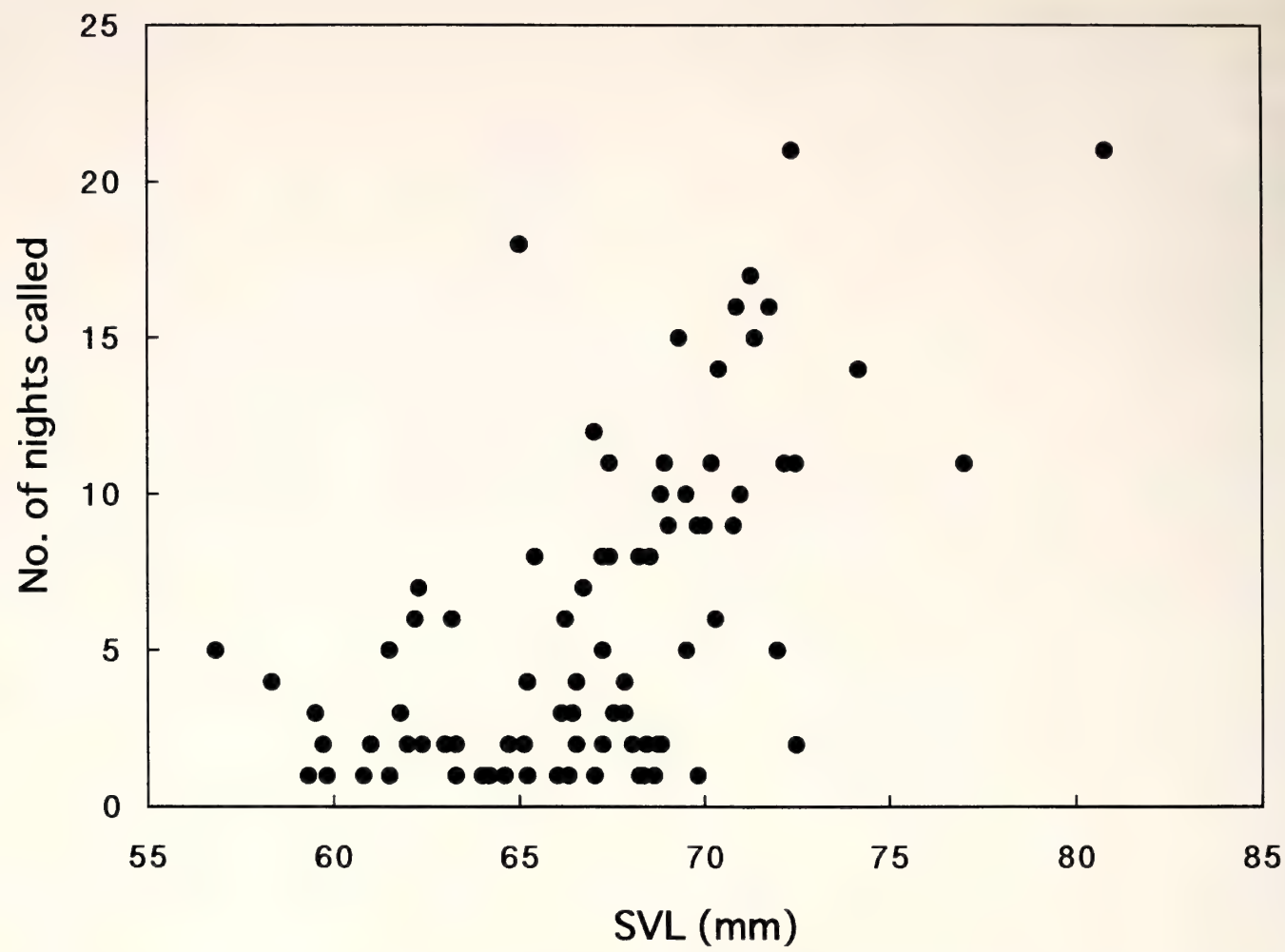


FIG. 9. Relationship between body size and the number of nights of calling in males of *R. nigromaculata* in 1990.

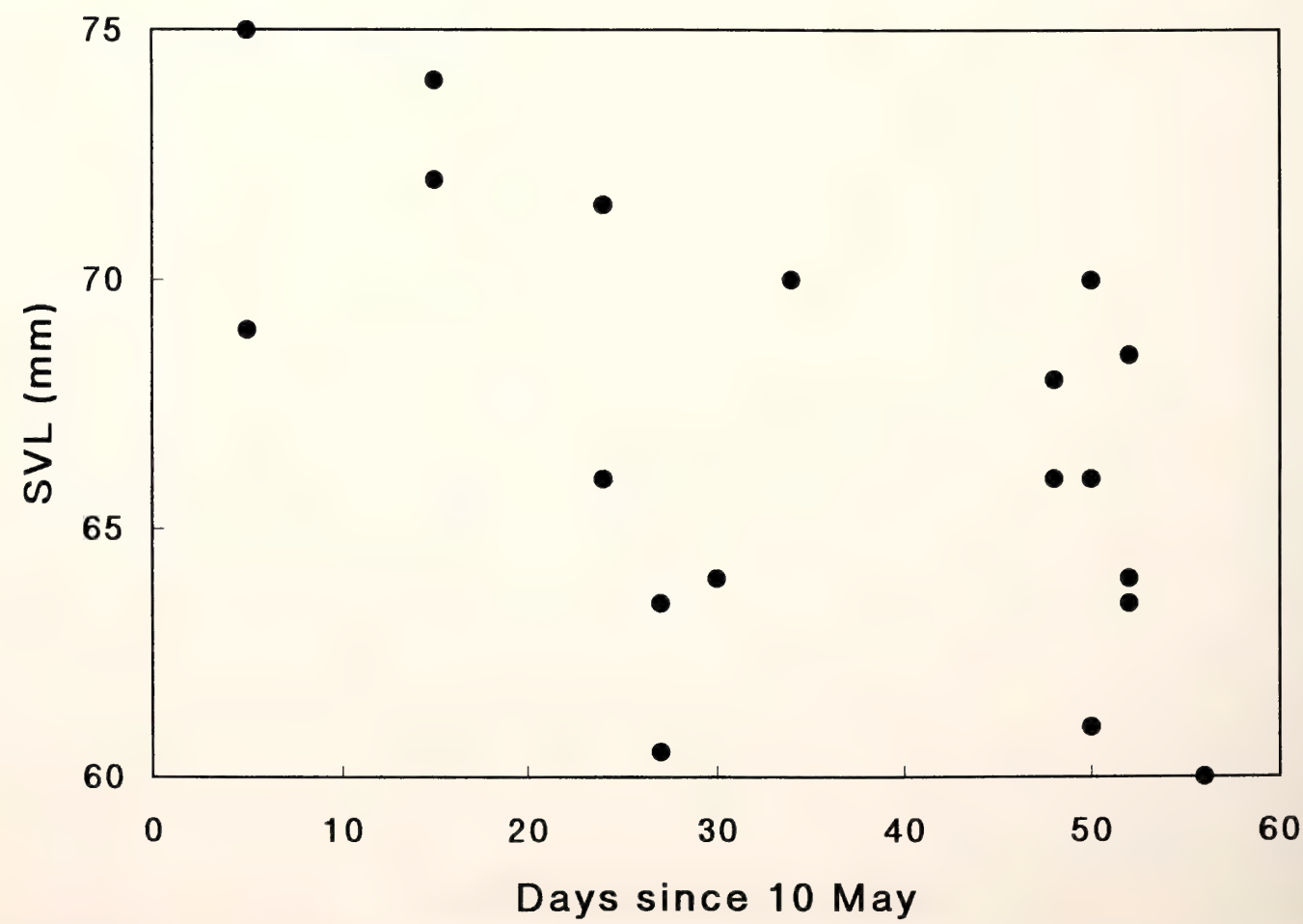


FIG. 10. Relationship between the date of oviposition and the female size of *R. nigromaculata* in 1990.

nificant tendency for larger females to spawn earlier than smaller females ($r = -0.566$, $N = 19$, $p = 0.012$). This tendency is consistent with the analyses of the female ovarian cycle of this species (see Shimoyama, 1993a).

During the course of the study, I found 30 conspecific pairs of *R. nigromaculata*. These pairs included individuals without markings. I observed the sequence of pair-formation in 13 cases. Of these, 10 (76.9%) were initiated by forced clasping by males. That is, calling males chased and clasped the females by force. In the remaining three cases, a gravid female visited a calling male and mated with the male. I could not obtain any evidence of active mate choice by females. No significant correlation was found between the male and female sizes in the amplexant pairs ($r = 0.046$, $N = 16$, $p > 0.8$; Fig. 11).

Heterospecific pair-formation and hybridization

I found a total of 12 heterospecific pairs. Of these, 11 (91.7 %) were composed of a male *R. nigromaculata* and a female *R. porosa brevipoda* (Shimoyama, 1999). Thus, remarkable asymmetry was observed in the combination of the heterospecific pairs. I observed the sequence of the heterospecific pair-formation for seven cases. In all of these heterospecific pairs, male *R. nigromaculata* dashed toward, chased, and clasped gravid females of *R. porosa brevipoda* which appeared in the mixed-species choruses. Most of the eggs deposited by these heterospecific pairs hatched normally (Shimoyama, 1999). So far as I observed in either species, no gravid female approached calling heterospecific males.

Figure 12 shows the relationship between the SVL of females and that of males in the heterospecific pairs. No significant correla-

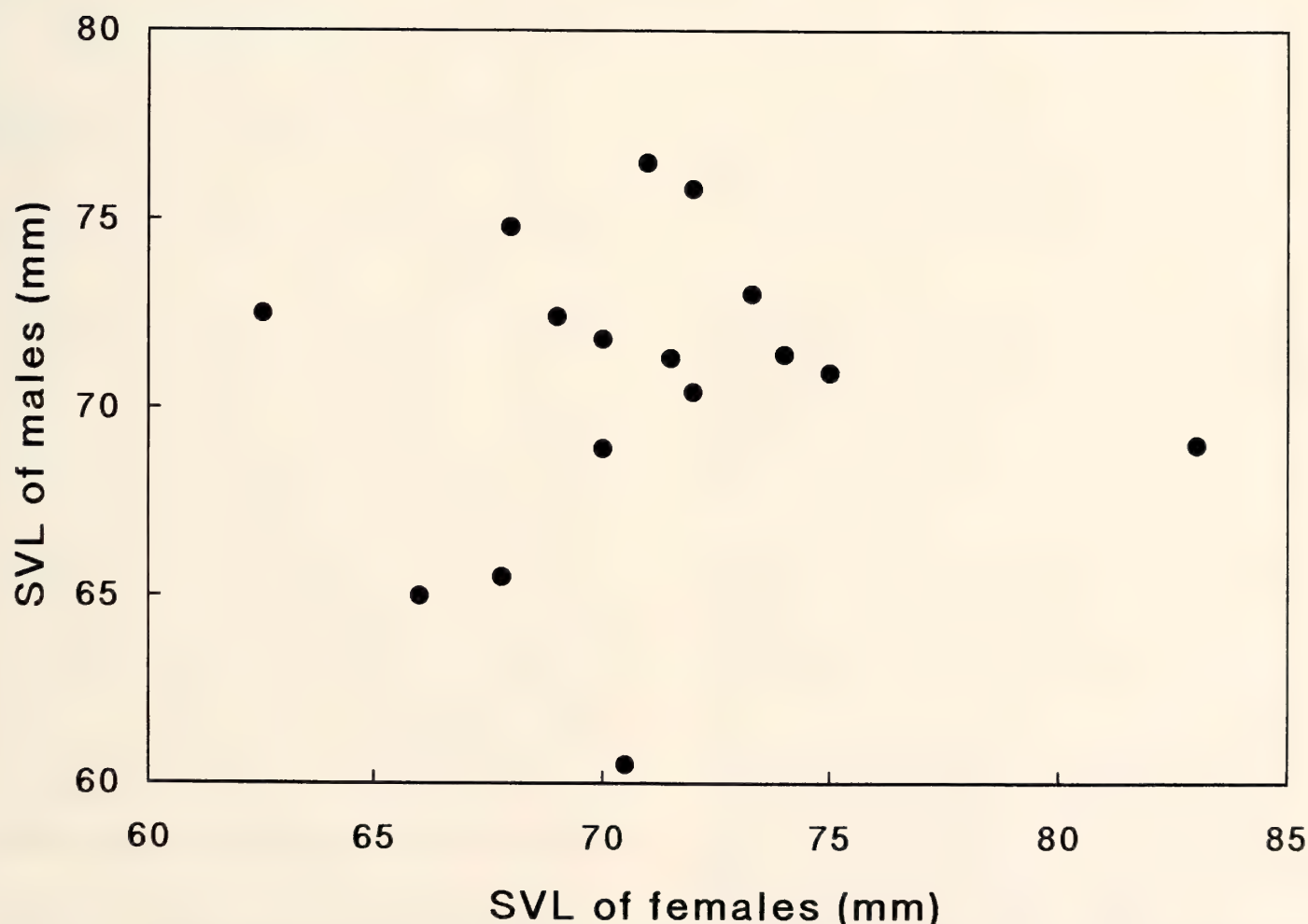


FIG. 11. Relationship between the male and female sizes in amplexant pairs of *R. nigromaculata*. Data from seven seasons were pooled.

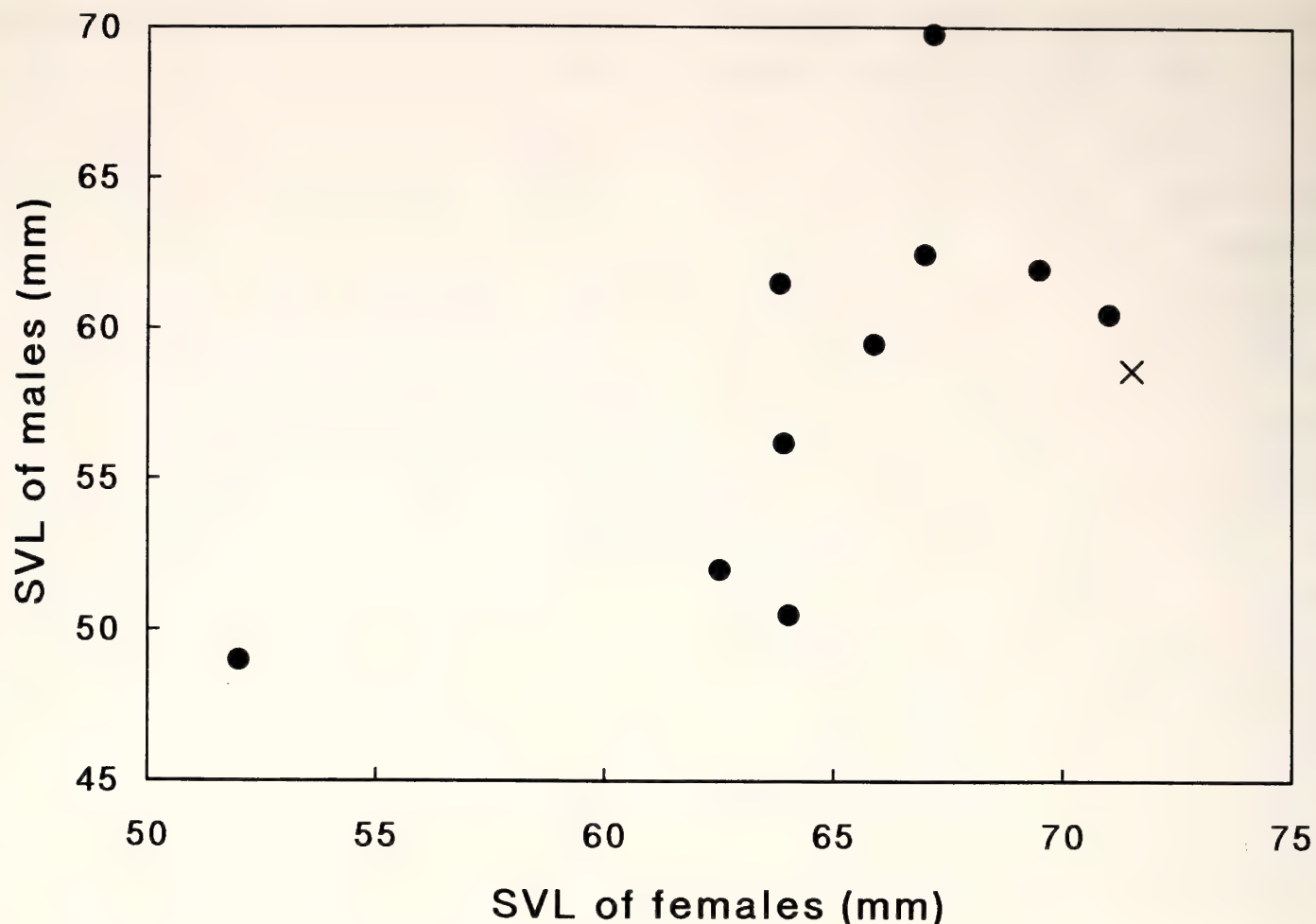


FIG. 12. Relationship between the male and female sizes in heterospecific pairs. Dots: pairs composed of a male *R. nigromaculata* and a female *R. porosa brevipoda*; cross: a pair composed of a male *R. porosa brevipoda* and a female *R. nigromaculata*.

tion was found between the size of male *R. nigromaculata* and that of female *R. porosa brevipoda* ($r=0.237$, $N=10$, $p>0.5$).

Speculation on the cause of the asymmetry in the heterospecific pairing and hybridization

As noted above, gravid females of neither species seemed to be attracted to the calling of heterospecific males. This implies that females of the two species were able to distinguish heterospecifics from conspecifics by auditory cues. Nevertheless, a total of 12 cases of heterospecific pairing and spawning were observed.

In my study site, both sexes of *R. porosa brevipoda* were numerically twice as abundant as those of *R. nigromaculata*. If heterospecific pairing occurs at random, the ratio of the heterospecific pairs with opposite combinations should be 1:1. However, distinct asymmetry was found in

the combination. Eleven of the 12 heterospecific pairs (91.7%) were composed of male *R. nigromaculata* and female *R. porosa brevipoda*. The ratio of the opposite combinations of the heterospecific pairs (11:1) was significantly different from the expected ratio (1:1; binomial test, $p=0.0063$). A similar tendency was also found under experimental conditions (Shimoyama, 1989). These tendencies suggest that the asymmetry in the combination of the heterospecific pairs was not affected by the difference in the number of individuals of the two species.

I will examine two ethological factors which might cause the asymmetry in the heterospecific pairings and hybridization.

- (1) Difference of pairing sequence between the two species

In *R. porosa brevipoda*, the majority of

pair-formations was initiated by females. This indicates that males tend to wait for the visit of females. On the other hand, forced clasping by males was the major pairing pattern in *R. nigromaculata* (10 out of the 13 cases). That is, male *R. nigromaculata* tended to dash toward and clasp any moving frog which appeared nearby. This distinct difference in the pairing sequence seems to be the most important factor causing the asymmetry in the heterospecific pairings.

(2) Difference of body size and shape

As shown in Figs. 1 and 7, both male and female *R. nigromaculata* were much larger than *R. porosa brevipoda*. In addition, both male and female *R. nigromaculata* can move more quickly than *R. porosa brevipoda*, because of the difference in body shape (see Maeda and Matsui, 1989). It seems probable that male *R. nigromaculata* could easily clasp gravid females of *R. porosa brevipoda*. On the other hand, male *R. porosa brevipoda* could not easily clasp female *R. nigromaculata*, because female *R. nigromaculata* are too large and nimble to be clasped by force.

Influence of hybridization on the two species

It is apparent that the costs of the hybridization for females are more serious than those for males. Females are forced to lose whole (or a half) of their gametes in the season by the hybridization, whereas loss of male gametes might be less. Hence, *R. porosa brevipoda* would suffer more serious costs than *R. nigromaculata* by the asymmetric hybridization. In other words, the asymmetric hybridization causes one-sided damage to the population of *R. porosa brevipoda*.

Because female hybrids between the two species are fertile (see Maeda and Matsui, 1989; Matsui, 1996), the female hybrids are able to produce offspring by means of backcrosses with males of either species.

Although I have no data on backcrosses, Nishioka et al. (1981, 1992) showed evidence of introgression between the two species inhabiting this basin. The introgression will also influence the decrease of the pure genes of *R. porosa brevipoda*.

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Ant Specialization in Diet of the Narrow-mouthed Toad, *Microhyla ornata*, from Amamioshima Island of the Ryukyu Archipelago

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Abstract: *Microhyla ornata* consumed numerous ants, representing 77.1% in number and 44.6% in volume of the diet. The toad took ants in higher proportion than were present in the surrounding environment, and therefore, could be viewed as an ant specialized predator. Ants were the most numerous prey in both spring and summer, while beetles and woodlice were less frequently taken in summer. Females have a larger body and wider mouth than males, and consumed significantly larger prey in maximum size than did males. However, mean prey size, and frequencies of occurrence for all prey taxa did not differ significantly between the sexes. These results suggest that the sexes do not differ in their use of food resources despite their morphological differences.

Key words: *Microhyla ornata*; Ant-specialists; Prey availability; Ryukyu Archipelago; Gape-limited predator

INTRODUCTION

The Anuran assemblage in the Ryukyu Archipelago (excepting Osumi Islands) is unique and more highly diversified than that found in the adjacent mainland of Japan. While 21 anuran species/subspecies occur in mainland Japan, 20 other species are distributed in the Ryukyu Archipelago, and 14 of them are endemic to the archipelago (Maeda and Matsui, 1999).

Quite a few comprehensive taxonomic and biogeographical studies have been made on anurans in the Ryukyu Archipela-

go (e.g., Matsui, 1994; Ota, 1998), but ecological studies are much more limited, and mostly confined to reproduction (e.g., Utsumiya, 1980, 1989). A preliminary report by Okochi and Katsuren (1989) is the only information available about diet of anurans on Okinawajima Island.

Microhyla ornata ranges throughout the Ryukyu Archipelago from Amamioshima southward through China to Southeast Asia and India (Frost, 1985), and inhabits various habitats from lowlands to montane regions (Maeda and Matsui, 1999). The toad is presumed to be specialized for eating ants or termites because of its small body and narrow mouth (Maeda and Matsui, 1999). However, detailed quantitative studies on the food habits of this species have not been done so far.

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Berry (1965) reported that ants predominated in the diet of two congeneric species, *M. butleri* and *M. heymonsii* from Singapore. Many dendrobatid and bufonid species are also well-known for eating numerous ants, and are called ant specialists because they take ants in higher proportion than are found in the environment (Toft, 1980, 1981).

Toft (1985), in reviewing resource partitioning studies in amphibians and reptiles, considered that food partitioning plays an important role in the organization of adult anuran communities. Therefore, knowledge of food habits of each community member might provide pivotal insights into the factors that are responsible for frog community structures in the Ryukyu Archipelago.

In order to obtain information on prey selection by the narrow-mouthed toad, *M. ornata*, we conducted field work on Amamioshima which is near the northern limits of its range.

MATERIALS AND METHODS

For the diet study, we collected toads in evergreen forests of the outskirts of Naze city on Amamioshima Is. (28°22' N, 129°31' E). We made collections at night (2100–2300 h) on 6 April (spring) and 12 July (summer) of 1998. We captured all individuals encountered on the forest floor, and immediately fixed them in 10% buffered formalin to preserve stomach contents with minimum digestion.

In order to estimate prey availability in the habitat of the toads, we sampled leaf litter invertebrates by two different methods. In spring, we set pit-fall traps near the temporal pool where toads were abundant. Fifteen plastic cups (90 mm in diameter and 130 mm in depth) were set in the ground at about 2 m intervals for two nights from 7 to 9 April. A small quantity of ethylene glycol was put into each cup to preserve samples. In summer, at the same

time as the collection of toads, we collected four 0.5 × 0.5 m quadrat samples of leaf litter and brought them to the laboratory. After large or inactive animals were removed from these samples, the remaining ones were extracted by Tullugren's funnel method (Aoki, 1973). These potential prey invertebrates were stored in ethylene glycol for later analyses.

Stomach contents were removed by dissection of toads, and preserved in 10% buffered formalin for later analyses. For each toad, snout-vent length (SVL) and mouth width (MW) were measured with a caliper to the nearest 0.1 mm.

We identified stomach contents and potential prey to the level of class or order except for Hymenoptera, which was classified into Formicidae and non-Formicidae. For holometabolous insects, larvae and adults were separately treated. The occurrence of plant materials or minerals was recorded for each stomach. Details about measurement of stomach contents are given in Hirai and Matsui (1999).

To detect seasonal variation in the diet, we compared the presence or absence of each prey taxon between spring and summer by Fisher's exact probability test. Next, we examined the relationships between prey availability and diet composition by calculating Kendall's rank correlation coefficients (τ). In this analysis, we used only taxa that were commonly found in both potential prey samples and the stomach contents because prey taxa found only in one of these might have large sampling errors. We presumed that the exclusion of these uncommon prey taxa would not affect the result of analyses because the common prey taxa accounted for more than 75% of both diet and prey samples. Moreover, we tested the sexual difference in the diet by comparing the presence or absence of each prey taxon by Fisher's exact probability test. In addition, we quantified dietary overlap between sexes by calculating simple similarity indices (Schoener,

1968):

$$C_{xy} = 1 - 0.5 \sum |P_{ix} - P_{iy}|$$

based on proportion of prey taxa (i) in diet of two different sexes (x and y). We calculated both numeric and volumetric overlap in this analysis.

RESULTS

Diet composition

We identified 1234 prey items extracted from 55 stomachs (41 males, 14 females) of 56 individuals captured; the remaining stomach from an immature female was empty. Prey items included five arthropod classes (Arachnida, Crustacea, Diplopoda, Chilopoda, and Insecta), of which Insecta contained eight orders (Table 1). Ants (Formicidae) were consumed by all individual toads with stomach contents, and predominated in the diet, representing 77.1% by number, and 44.6% by volume. The next most frequently consumed prey taxon was beetles (Coleoptera; 67.3%), but they made up only 6.4% by number, and 16.9% by volume. The other prey taxa constituted minute fractions, making up less than 5% by number, and 15% by volume. Plant (vegetable scraps) and mineral materials (pebbles and dirt) occurred in 47.3% and 1.8%, respectively, of the stomachs.

Seasonal variation in diet

The toads appeared to take more prey in spring (Mean ± SE = 26.1 ± 3.9) than in summer (16.5 ± 2.7), but the difference was not significant (U-test, p > 0.05). However, the volume of stomach contents differed seasonally, and almost three times more food was ingested in spring (59.2 ± 8.1 mm³) than in summer (20.8 ± 4.0 mm³) (U-test, p < 0.01).
Ants constituted the bulk of the diet numerically and volumetrically, and their proportions varied little seasonally (Table 2). Among 10 prey taxa commonly consumed in both seasons, all prey taxa except

TABLE 1. Diet composition (in %) of *Microhyla ornata* (1234 prey from 55 individuals, total volume 2448.3 mm³). Abbreviations: F=frequency of occurrence; N=numeric proportion; V=volumetric proportion

Prey taxa	F	N	V
Insecta			
Hymenoptera			
Formicidae	100.0	77.1	44.6
non-Formicidae	5.5	0.2	0.5
Coleoptera	67.3	6.4	16.9
larvae	3.6	0.3	4.1
Diptera	14.6	1.0	0.8
larvae	10.9	1.0	4.4
Lepidoptera	1.8	0.2	0.2
larvae	1.8	<0.1	0.9
Hemiptera	20.0	1.1	3.2
Isoptera	3.6	4.7	1.4
Orthoptera	1.8	<0.1	0.3
Collembola	21.8	1.5	0.2
Arachnida			
Araneae	10.9	0.7	1.1
Pseudoscorpiones	1.8	<0.1	<0.1
Acarina	21.8	2.5	0.5
Crustacea			
Isopoda	20.0	1.3	11.3
Chilopoda	12.7	0.7	2.6
Diplopoda	21.8	1.2	7.1
Plant materials	47.3	—	—
Minerals	1.8	—	—

for ants and wasps (non-formicids) were found more frequently in spring than in summer, but only two prey taxa, beetles and woodlice (Isopoda), differed significantly in frequency of occurrence (Fisher's exact probability test, p < 0.01 for Coleoptera; p < 0.05 for Isopoda). Volumetric contribution by these two prey taxa was great in spring, but it showed striking decrease in summer. By contrast, termites (Isoptera) made up a larger numerical proportion in summer because of two individuals (9.5% in frequency) that contained unusually many termites (Table 2). Frequency of oc-

TABLE 2. Dietary comparison of *Microhyla ornata* between spring (887 prey from 34 individuals, total volume 2012.4 mm³) and summer (347 prey from 21 individuals, total volume 435.9 mm³). See Table 1 for abbreviations.

Prey taxa	F		N		V	
	Spring	Summer	Spring	Summer	Spring	Summer
Insecta						
Hymenoptera						
Formicidae	100.0	100.0	79.8	70.0	45.0	43.1
non-Formicidae	2.9	9.5	0.1	0.6	0.3	1.6
Coleoptera	82.4	42.9	7.7	3.2	18.4	9.5
larvae	0	9.5	0	1.2	0	23.2
Diptera	17.7	9.5	1.1	0.6	0.7	1.0
larvae	17.7	0	1.4	0	5.4	0
Lepidoptera	0	4.8	0	0.6	0	1.0
larvae	2.9	0	0.1	0	1.1	0
Hemiptera	23.5	14.3	0.9	1.4	3.4	2.5
Isoptera	0	9.5	0	16.7	0	7.7
Orthoptera	0	4.8	0	0.3	0	1.7
Collembola	26.5	14.3	1.7	0.9	0.2	0.1
Arachnida						
Araneae	14.7	4.8	0.8	0.3	1.3	<0.1
Pseudoscorpiones	0	4.8	0	0.3	0	<0.1
Acarina	26.5	14.3	2.6	2.3	0.3	1.4
Crustacea						
Isopoda	29.4	4.8	1.6	0.6	12.8	4.0
Chilopoda	20.6	0	1.0	0	3.2	0
Diplopoda	26.5	14.3	1.2	1.2	7.8	3.4
Plant materials	26.5	90.5	—	—	—	—
Minerals	2.9	0	—	—	—	—

currence of plant materials markedly increased from spring (26.5%) to summer (90.5%), and differed significantly between the seasons (Fisher’s exact probability test, $p<0.01$).

Prey selection

A total of 20 potential prey invertebrates were collected when samples in spring and summer were combined (Table 3). Among these, larval caddisflies (Trichoptera), earwigs (Dermaptera), gastropod snails, and earthworms (Oligochaeta) were not found in the toad stomachs. Conversely, moths (Lepidoptera) and their larvae were not

sampled from the surrounding habitat. Specifically in spring, 11 of 14 potential prey invertebrates sampled from the environment were found in diet composition. Ants seemed to be the most readily available prey for toads, and were consumed in much higher proportion than those found in the environment. However, the next most abundant possible prey, collembolans, were consumed much less in proportion by toads. Consequently, there was no significant correlation between prey availability in the habitat and diet composition ($\tau=0.278$, $p>0.05$). In summer, 12 of 17 potential prey invertebrate taxa were actually found

TABLE 3. Comparison of diet composition of *M. ornata* with prey availability in the environment assessed by pitfall traps in spring and funnel traps in summer.

Prey taxa	Spring				Summer			
	Diet		Environment		Diet		Environment	
	n	%	n	%	n	%	n	%
Formicidae	708	79.8	97	41.6	243	70.0	40	10.6
non-Formicidae	1	0.1	1	0.4	2	0.6	0	0
Coleoptera	68	7.7	10	4.3	11	3.2	4	1.1
larvae	0	0	0	0	4	1.2	15	4.0
Diptera	10	1.1	26	11.2	2	0.6	4	1.1
larvae	12	1.4	1	0.4	0	0	1	0.3
Lepidoptera	0	0	0	0	2	0.6	0	0
larvae	1	0.1	0	0	0	0	0	0
Trichoptera larvae	0	0	0	0	0	0	1	0.3
Hemiptera	8	0.9	2	0.9	5	1.4	2	0.5
Isoptera	0	0	6	2.6	58	16.7	8	2.1
Dermaptera	0	0	0	0	0	0	1	0.3
Orthoptera	0	0	0	0	1	0.3	1	0.3
Collembola	15	1.7	35	15.0	3	0.9	1	0.3
Araneae	7	0.8	17	7.3	1	0.3	2	0.5
Pseudoscorpiones	0	0	1	0.4	1	0.3	0	0
Acarina	23	2.6	3	1.3	8	2.3	161	42.8
Isopoda	14	1.6	11	4.7	2	0.6	35	9.3
Chilopoda	9	1.0	0	0	0	0	1	0.3
Diplopoda	11	1.2	17	7.3	4	1.2	11	2.9
Gastropoda	0	0	6	2.6	0	0	0	0
Oligocheata	0	0	0	0	0	0	88	23.4

in stomachs of toads. As was found in spring, the proportion of ants in the diet was larger than that found in the environment. Instead, mites, the most abundant prey in the environment, were consumed by few toads. We could not detect a significant correlation between prey availability and diet composition ($\tau=0.381$, $p>0.05$).

Comparisons between sexes

Females (mean \pm SE = 30.5 ± 0.6 mm, range = 25.6–33.0 mm) were significantly larger in SVL than males (26.3 ± 0.2 mm, 22.1–29.6 mm) (U-test, $p<0.01$). Females also had a significantly wider mouth (8.3 ± 0.1 mm, 7.5–9.3 mm) than males (7.3 ± 0.1 mm, 6.1–8.5 mm) ($p<0.01$). Sexual

difference was highly significant in maximum prey size (females: 19.9 ± 4.1 mm; males: 12.0 ± 2.2 mm) ($p<0.01$), but was not significant in mean (females: 3.0 ± 0.5 mm, males: 1.9 ± 0.2 mm) or minimum prey size (females: 0.6 ± 0.2 mm, males: 0.3 ± 0.04 mm) ($p>0.05$ for both) due to their large variations. Similarly, neither the number (females: 28.7 ± 8.0 , males: 20.3 ± 2.4) nor the volume (females: 66.8 ± 16.8 mm³, males: 36.9 ± 4.8 mm³) of stomach contents differed significantly between the sexes ($p>0.05$ for both).

Diet compositions did not differ markedly between females and males as suggested by high dietary overlap (similarity indices = 0.85 in number and 0.70 in volume:

TABLE 4. Dietary comparison of female (402 prey from 14 individuals, total volume 934.6 mm³) and male (832 prey from 41 individuals, total volume 1513.7 mm³) *Microhyla ornata*. See Table 1 for abbreviations.

Prey taxa	F		N		V	
	Female	Male	Female	Male	Female	Male
Insecta						
Hymenoptera						
Formicidae	100.0	100.0	86.1	72.7	57.8	36.5
non-Formicidae	7.1	4.9	0.3	0.2	0.7	0.5
Coleoptera	57.1	70.7	3.0	8.1	9.3	21.5
larvae	7.1	2.4	0.3	0.4	2.6	5.1
Diptera	7.1	17.1	0.3	1.3	0.9	0.7
larvae	21.4	7.3	2.2	0.4	8.0	2.2
Lepidoptera	0	2.4	0	0.2	0	0.3
larvae	7.1	0	0.3	0	2.4	0
Hemiptera	14.3	22.0	0.8	1.2	1.5	4.3
Isoptera	0	4.9	0	7.0	0	2.2
Orthoptera	0	2.4	0	0.1	0	0.5
Collembola	7.1	26.8	0.5	1.9	<0.1	0.2
Arachnida						
Araneae	21.4	7.3	1.0	0.5	1.7	0.8
Pseudoscorpiones	7.1	0	2.0	0	<0.1	0
Acarina	42.9	14.6	0.3	2.8	0.3	0.6
Crustacea						
Isopoda	14.3	22.0	1.0	1.4	8.4	13.1
Chilopoda	14.3	12.2	0.8	0.7	1.2	3.5
Diplopoda	28.6	19.5	1.5	1.1	5.3	8.2
Plant materials	42.9	53.7	—	—	—	—
Minerals	7.1	0	—	—	—	—

Table 4). Indeed, frequency of occurrence of all prey taxa did not differ significantly between the sexes (Fisher's exact probability test, $p > 0.05$ for all prey taxa). Ants predominated in their diet, both numerically and volumetrically (Table 4).

DISCUSSION

Ants were consumed by all individuals of *M. ornata* studied, and comprised more than 70% of the total prey items. Following Simon and Toft's (1991) definition, *M. ornata* is regarded as an ant specialist because they consumed ants higher in propor-

tion than those available from the environment.

Berry (1965) reported that ants occurred in more than 94.9% of stomachs, and made up 86–87% of the total prey items in the diet of two microhylids, *M. butleri* and *M. heymonsii* from Singapore. Results of our study in *M. ornata*, i.e., ants occurring in all stomachs, and making up 77.1% of total prey items, are consistent with Berry's (1965) observation. Therefore, the food habits of this genus would be characterized by eating numerous ants.

Seasonal variation in diet was conspicuous in beetles and woodlice. In spring,

these prey taxa were consumed more frequently, and made up relatively large proportions by volume. Fewer chances of consuming these prey might have caused a decrease in the amount of food ingested during summer. Instead, plant materials were found more frequently in summer. A ranid frog, *Rana hexadactyla*, has been reported to supplement energy gain by consuming plant materials (Das, 1996), and *M. ornata* might also consume plant materials frequently in summer so as to compensate for reduction of animal food consumption.

Sexual dimorphism in body size and mouth width was observed in *Microhyla ornata*, and both the variables were larger in females than in males. Generally, anurans are gape-limited in predation, and size of consumable prey is regulated by body size or mouth width (e.g., Kramek, 1972; Toft, 1980). Hence, such sexual dimorphism in morphology may help to alleviate potential competitive interactions by partitioning food resources. In fact, females of *Rana cancrivora* with larger body and wider mouth than males were reported to consume larger prey than males (Premo and Atmowidjojo, 1987). In our observations, where maximum prey size was larger in females suggests that consumable prey size in *M. ornata* is also determined by the mouth width. However, the sexes did not differ in mean or minimum prey size. Further, they were similar in frequency of occurrence for all prey taxa. These lines of evidence indicate that food resources were not partitioned between the sexes. High dietary overlap of females and males also seems to support this assumption.

Among anurans occurring in the Ryukyu Archipelago, diet data for four ranid species, *Rana namiyei*, *R. narina*, *R. ishikawae*, and *R. holsti* from Okinawajima Island, are available (Okochi and Katsuren, 1989). These four species are relatively large, reaching 55 to 120 mm in SVL, and consume few ants. Therefore, ant specialization in *M. ornata* of less than 30 mm in

SVL might be partially responsible for the coexistence with larger anuran species. Anuran fauna in the subtropical Ryukyu Archipelago is characterized by the presence of many endemic species, and by greater species diversity than in temperate mainland Japan (Matsui, 1996).

In the tropics where species diversity is greater than in temperate and subtropical regions, and anurans have specialized their various ecological attributes (Duellman and Trueb, 1986), diet specialization has been demonstrated to be important in structuring an anuran community (Toft, 1980). Therefore, ant specialization in *M. ornata* might be playing a great role in structuring anuran communities in the Ryukyu Archipelago as well. Further studies based on community ecology are necessary to evaluate the importance of the role of food specialization.

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Nomenclatural History and Rediscovery of *Rhacophorus lateralis* Boulenger, 1883 (Amphibia: Rhacophoridae)

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Abstract: The poorly known south-west Indian rhacophorid, *Rhacophorus lateralis* Boulenger, 1883, known from a unique holotype in the BMNH, is redescribed based on two adult females, from South Coorg, Karnataka State, south-western India. The species is listed in recent lists as valid, despite an attempted synonymy with *Rhacophorus malabaricus* Jerdon, 1870, by Wolf (1936). The species is diagnosed by the following suite of characters: skin of forehead free; dorsum dark brown with a pair of yellow lines that run from the region around the nostrils, over the eyelids, along the sides of the body, terminating in the inguinal region; small adult body size (SVL to 32.8 mm). The species is illustrated for the first time.

Key words: *Rhacophorus lateralis*; Nomenclatural history; Rediscovery; Redescription; Western Ghats; India

INTRODUCTION

Rhacophorus lateralis was described by Boulenger (1883) based on a unique holotype (BMNH 82.2.10.75) from “Malabar” (at present in southern Kerala State, south-western India) that was collected by Colonel Richard Henry Beddome. No further specimens of this species have come to light since the original description, despite numerous surveys of the hill ranges of southern India that are referred to as the Western Ghats (see Mani, 1974, for a description and Dutta, 1997, for a bib-

liography of published works on amphibians of the region). Several workers listed the species as valid: Boulenger (1890: 473); Ahl (1931: 165, as *Rhacophorus* [*Rhacophorus*] *lateralis*); Inger and Dutta (1986), Daniel and Sekar (1989), Daniels (1992), Dutta (1997: 102), and Das and Dutta (1998). On the other hand, the species is not listed by Gorham (1974), Frost (1985), Dubois (1986), or Duellman (1993), perhaps following Wolf (1936: 214) who considered it as possibly synonymous with *Rhacophorus reinwardtii malabaricus* (= *R. malabaricus* Jerdon, 1870). The taxon has never been illustrated.

The collection of two adult females and an unsexed metamorph assignable to *Rhacophorus lateralis* Boulenger, 1883,

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from Lakunda Estate (12°03' 18" N; 76°02' 62" E), Virajpet District, Srimangala Nadu, South Coorg (Kodagu) District, Karnataka State, south-western India, provides the opportunity to redescribe the species on the basis of the two adult females, illustrate one of these, and announce the rediscovery of the species after over a century.

METHODS

Measurements were taken with a Mitutoyo™ dial caliper (to the nearest 0.1 mm) from specimens preserved in ethanol. The following measurements were taken: snout-vent length, SVL (from tip of snout to vent); tibia length, TBL (distance between surface of knee to surface of heel, with both tibia and tarsus flexed); axilla to

groin distance, A-G (distance between posterior edge of forelimb at its insertion to body to anterior edge of hind limb at its insertion to body); head length, HL (distance between angle of jaws and snout-tip); head width, HW (measured at angle of jaws); head depth, HD (greatest transverse depth of the head, taken posterior to orbital region); eye diameter, ED (diameter of the orbit); eye to tympanum distance, E-T (distance between posterior-most point of orbit and anterior-most edge of tympanum); upper eyelid width, UE (greatest width of upper eyelid); interorbital width, IO (least distance between upper eyelids); internarial distance, IN (distance between nostrils); eye to snout-tip distance, E-S (distance between anterior-most point of orbit to tip of snout); eye to nostril distance, E-N (distance be-



FIG. 1. Adult female *Rhacophorus lateralis* (ZSI A9071). Marker = 15Q12mm.

tween anterior-most point of orbit and nostril); horizontal tympanum diameter, HTYD (diameter of left tympanum, taken across hozontal plane); vertical tympanum diameter, VTYD (diameter of left tympanum, taken across vertical plane); and diameter of disk on Finger III, FIIID (greatest width of terminal disk on Finger III). Lengths of digits were measured from the base of the web to the distal tips of the digits. Institutional abbreviations follow Leviton et al. (1985).

REDESCRIPTION OF *RHACOPHORIS*
LATERALIS BOULENGER, 1883

Redescription (based on ZSI A9071-72, both adult females)

SVL 29.5 and 32.8 mm (subsequent ratios in the same sequence); habitus slender (Fig. 1); head relatively short (HL/SVL ratio 0.27 and 0.26) and broad (HW/SVL ratio 0.31 and 0.30), its width greater than its length (HW/HL ratio 1.11 and 1.16); snout short, obtusely pointed, projecting a little beyond level of mandibles; nostril closer to tip of

snout than to eye (E-N/E-S ratio 0.58 and 0.57). Canthus rostralis flattened in transverse section; lores slightly concave. Eyes large (ED/HL ratio 0.63 and 0.49); eye diameter greater than eye-nostril distance (ED/E-N ratio 2.22 and 1.64); interorbital distance greater than width of upper eyelid (IO/UE ratio 1.59 and 2.64). Supratympanic fold absent. Tympanum distinct, its diameter greatest vertically (HTYD/VTYD ratio 0.94 and 0.80), less than eye diameter (HTYD/ED ratio 0.29 and 0.39), situated posteroventrally to level of eyes, at a little distance from the posterior corner of the eyes. Nostrils dorsolaterally oriented, oval. Vomerine teeth in two short, oblique series between choanae. Inner margin of mandible with a weak w-shaped notch anteriorly. Choanae oval, separated from each other by a distance over five times greater their width. Tongue large, smooth, lacking median conical lingual papilla, elongate, bifid and free posteriorly.

Forelimbs relatively short; tips of fingers dilated into large, flattened, rounded disks (Fig. 2a) with circummarginal grooves; the

TABLE 1. Morphometric data (in mm) on two adult female *Rhacophorus lateralis* from Karnataka, south-western India (see text for details).

	ZSI A9071	ZSI A9072
Snout-vent length	29.5	32.0
Axilla-groin distance	14.1	18.0
Head length	8.1	8.3
Head width	9.0	9.6
Head depth	5.6	5.3
Eye diameter	5.1	4.1
Upper eyelid width	2.2	2.2
Interorbital distance	3.5	5.8
Internarial distance	2.8	2.5
Eye-snout-tip distance	4.0	4.4
Eye-nostril distance	2.3	2.5
Eye-tympanum distance	0.6	0.7
Tibia length	16.3	17.8
Disk diameter finger III	1.5	1.5
Horizontal tympanum diameter	1.5	1.6
Vertical tympanum diameter	1.6	2.0

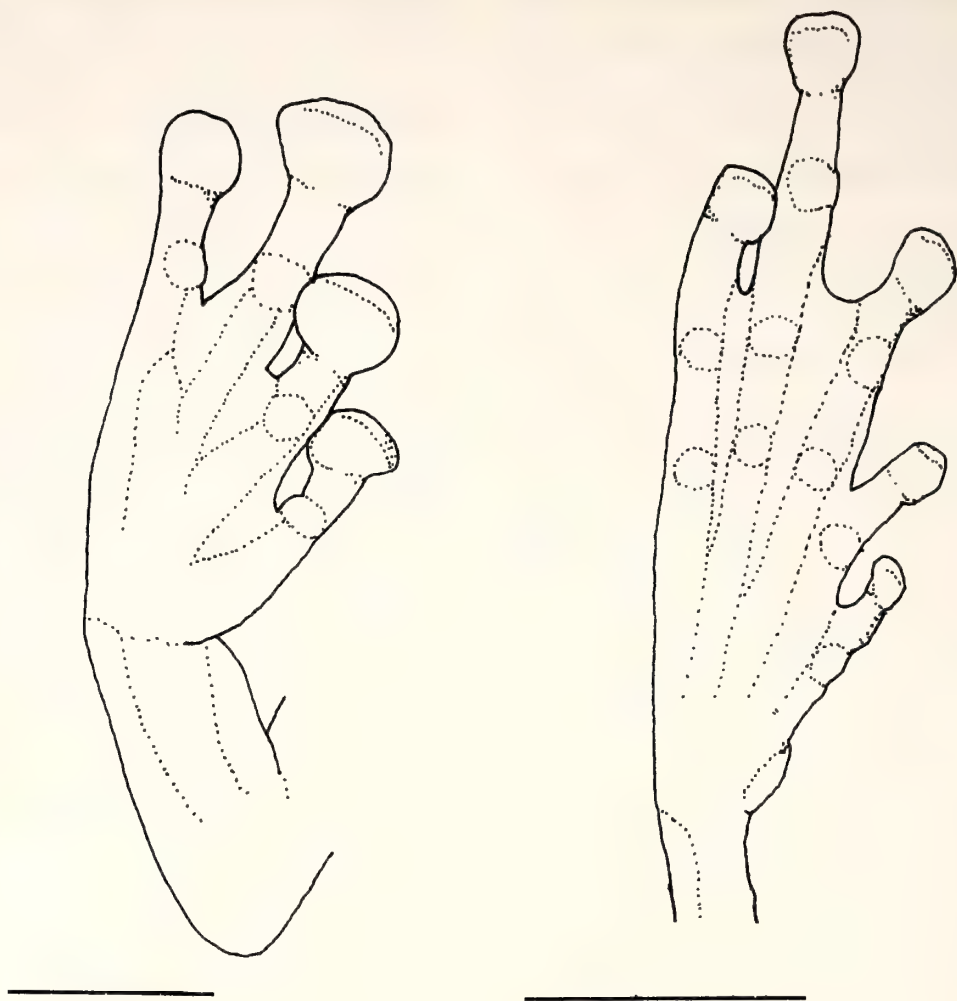


FIG. 2. Fore and hind limb of *Rhacophorus lateralis* (ZSI A9071), showing subarticular tubercles on venter of hand (2a), and on ventral surface of foot (2b). Markers = 5 mm.

largest disk (on Finger III) approximately equal to horizontal diameter of tympanum (FIID/HTYD ratio 1.00 and 0.94). Fingers broadly webbed, most extensive webbing between Fingers I and II not reaching proximal subarticular tubercles. All fingers with dermal fringes on inner and outer aspects. No dermal fringe on elbow. Relative lengths of fingers: 3 > 4 > 2 > 1. Hind limbs relatively long; tibia long (TBL/SVL ratio 0.55 and 0.56); tips of toes dilated into flattened disks (Fig. 2b) with

circummarginal groove, are slightly smaller than those on fingers. Webbing on Toe I between distal subarticular tubercle and digit tip; on Toe II to base of disks on outer edge and to slightly beyond distal subarticular tubercle on inner edge; on Toe III to base of disks on outer edge and to beyond distal subarticular tubercle on inner edge; on Toe IV, to base of disk on outer edge and to the distal subarticular tubercle, as a broad web, reaching the tip as a narrow sheath in the inner; and on Toe V, to base

TABLE 2. Lengths of digits (in mm) of left hand and foot of two adult female *Rhacophorus lateralis* from Karnataka, south-western India (see text for details).

	Fingers				Toes				
	1	2	3	4	1	2	3	4	5
ZSI A9071	3.4	4.4	6.4	6.0	3.4	5.0	8.0	10.8	8.7
ZSI A9072	4.2	4.9	6.8	6.2	3.7	5.6	8.5	12.3	9.7

of disks. Toes I and V have dermal fringes on outer edges. Distinct, elongated inner metatarsal tubercle, outer metatarsal tubercle absent. Relative lengths of toes: $4 > 5 > 3 > 2 > 1$.

Dorsum smooth, lacking tubercles. Outer edge of upper eyelids faintly granular. Surface of throat, pectoral and abdominal regions smooth. Undersurface of forelimbs and the undersurface of thighs smooth; trailing edge of hind limbs without rounded tubercles. Cloacal opening directed posteroventrally, close to lower level of thighs.

Colouration (in preservative)

ZSI A9071 has a dark chestnut dorsum, with a pale yellow stripe that commences from above the nostrils, running over the upper eyelids, to over the tympanum and along the sides of the body, terminating in the inguinal region. Upper surfaces of thigh and shank with narrow dark brown stripe, bounded by more extensive areas of pale yellow. Dorsum patterned with fine dark dots. Forearm and thigh with narrow dark brown bars on the dorsum. Upper lip, upper arm, digits, undersurface of limbs and body unpatterned pale yellow. The posterior surface of thigh unpatterned yellow. The heel with a pale yellow, sinuous marking. In life, the field notes of the collectors indicate that the frogs were green dorsally, dotted with blue. ZSI A9072-73 are discoloured, although both show the pale lateral stripes and the finely dark-banded dorsal surface of limbs.

Size variation and other notes

The SVL of the holotype was reported to be 31 mm in the original description, while the two adult females being reported here are 29.5 and 32.8 mm; the smallest individual (ZSI A9073, a metamorph with a pale lateral stripe and the vestige of a tail) is 16.7 mm. Other measurements are in Tables 1-2. No males are represented in the present series, and the small size of the adult



FIG. 3. Radiograph of adult female *Rhacophorus lateralis* (ZSI A9071).

females (with developed ovaries) is remarkable among members of the genus. Skin over the cranium is free, not coossified to frontoparietal, nasal, or squamosal bones. Figure 3 shows radiographs of ZSI A9071.

Comparisons

Because the species has been considered synonymous with *Rhacophorus malabaricus* by some workers, *R. lateralis* is here compared with congeners from the Western Ghats. Only opposing suites of characters are listed. *R. malabaricus* Jerdon, 1870: dorsum green, lacking light lateral stripes; digits webbed to disks; and SVL to 48.3 mm; *R. calcadensis* Ahl, 1927, dorsum reddish-brown, lacking light lateral stripes; digits webbed to disks; tympanum 40% eye diameter; and SVL to 48.3 mm; *R. pseudomalabaricus* Vasudevan and Dutta, 2000, dorsum green, lacking light lateral stripes; tympanum indistinct; head length equals width; and SVL 66.8 mm; and *R. lateralis* Boulenger, 1883; dorsum brown, lacking distinct light lateral stripes; and tympanum half eye diameter.

COLLECTION NOTES

The adult females were collected on 19 and 29 July 1998. The metamorph was discovered on a leaf above a tank on 3 August 1998. The locality is a moist evergreen forest within Lakunda Estate, Virajpet District, Srimangala Nadu, South Coorg (Kodagu) District, Karnataka State, southwestern India. The discovery of the metamorph in the month of August indicates that the species breeds during the summer (Southwest) Monsoons. *Rhacophorus malabaricus* was taken alongside *R. lateralis* at this locality.

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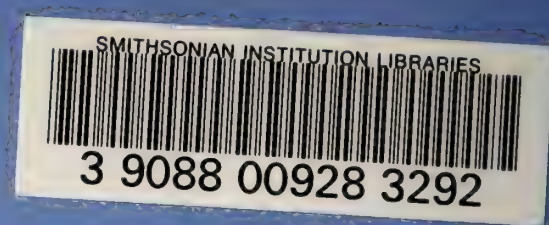
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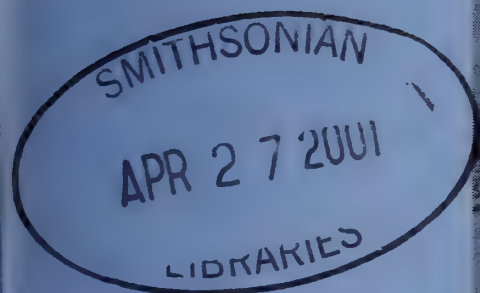
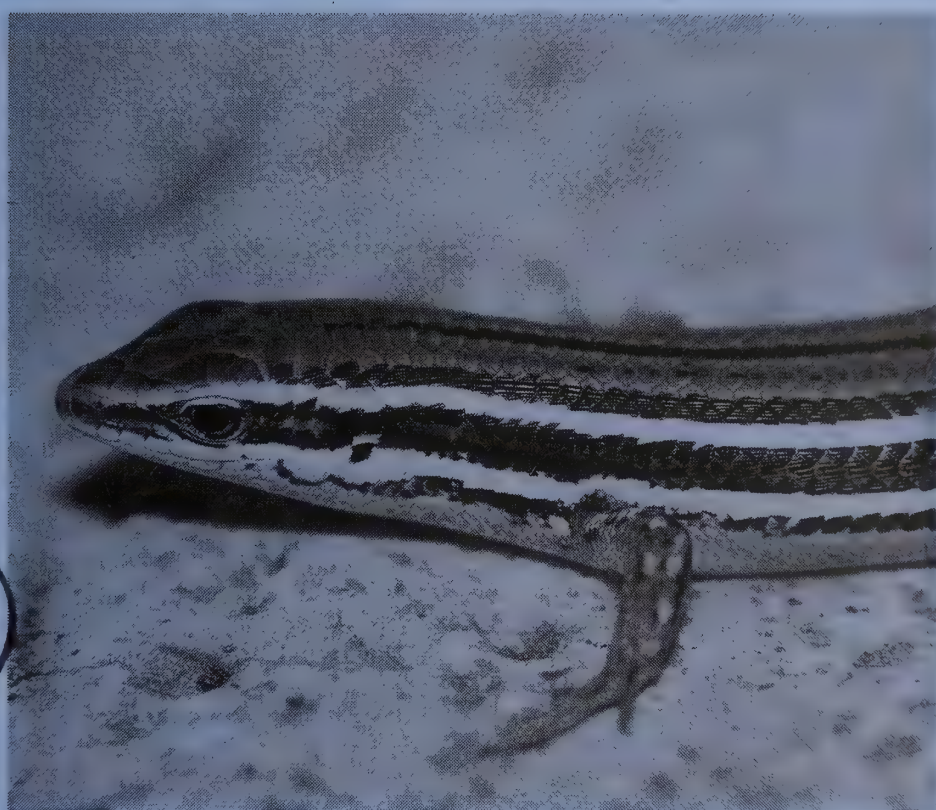
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Cover Illustration: *Mabuya cumingi* first recorded from Lanyu Island, Taiwan. A photograph taken by Hidetoshi Ota.

Phylogenetic Position of *Draco fimbriatus*, with a Molecular Perspective on the Historical Biogeography of the Genus *Draco* (Reptilia: Agamidae)

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Abstract: The phylogenetic relationship of *Draco fimbriatus* with other congeneric species was inferred from 848 base pairs of the mitochondrial 12S and 16S rRNA genes. The results confirmed that the closest affinity was between *D. fimbriatus* and *D. maculatus*. Our results also suggested that *D. lineatus* diverged first, followed by the *D. cornutus*—*D. volans* cluster, *D. dussumieri*, the *D. fimbriatus*—*D. maculatus* cluster, and *D. blanfordii* in that order, leaving *D. obscurus* and *D. taeniopterus* as monophyletic. The taxonomic diversity of *Draco* in each area of Southeast Asia appears to have increased through multiple colonizations rather than through *in situ* diversifications.

Key words: Agamidae; *Draco*; Mitochondrial DNA; Phylogenetics

INTRODUCTION

The genus *Draco* Linnaeus, 1758, consisting of some 21 species, is one of the most prominent genera of the family Agamidae, characterized by the presence of a patagium (a wing-like skin extension supported by elongated ribs) along the flanks. This genus is distributed in southern India and throughout Southeast Asia (Inger, 1983; Musters, 1983; Lazell, 1987, 1992; Ross and Lazell, 1990). Musters (1983), on the basis

of cluster analysis of a distance matrix for morphological characters, recognized two major lineages within this genus that were distinguished most simply by the direction of the nostrils—outward or upward (Fig. 1). Based on the DNA sequence data, Honda et al. (1999b) negated this view, and recognized at least four distinct lineages within the genus. Their analyses, however, failed to resolve the relationships of these major lineages in detail.

Honda et al. (1999b) did not investigate nine species of *Draco*. Of these, *D. mindanensis*, *D. spilopterus*, and six recently described or revalidated species (Lazell, 1987, 1992; Ross and Lazell, 1990) were nevertheless surmised to be closely related

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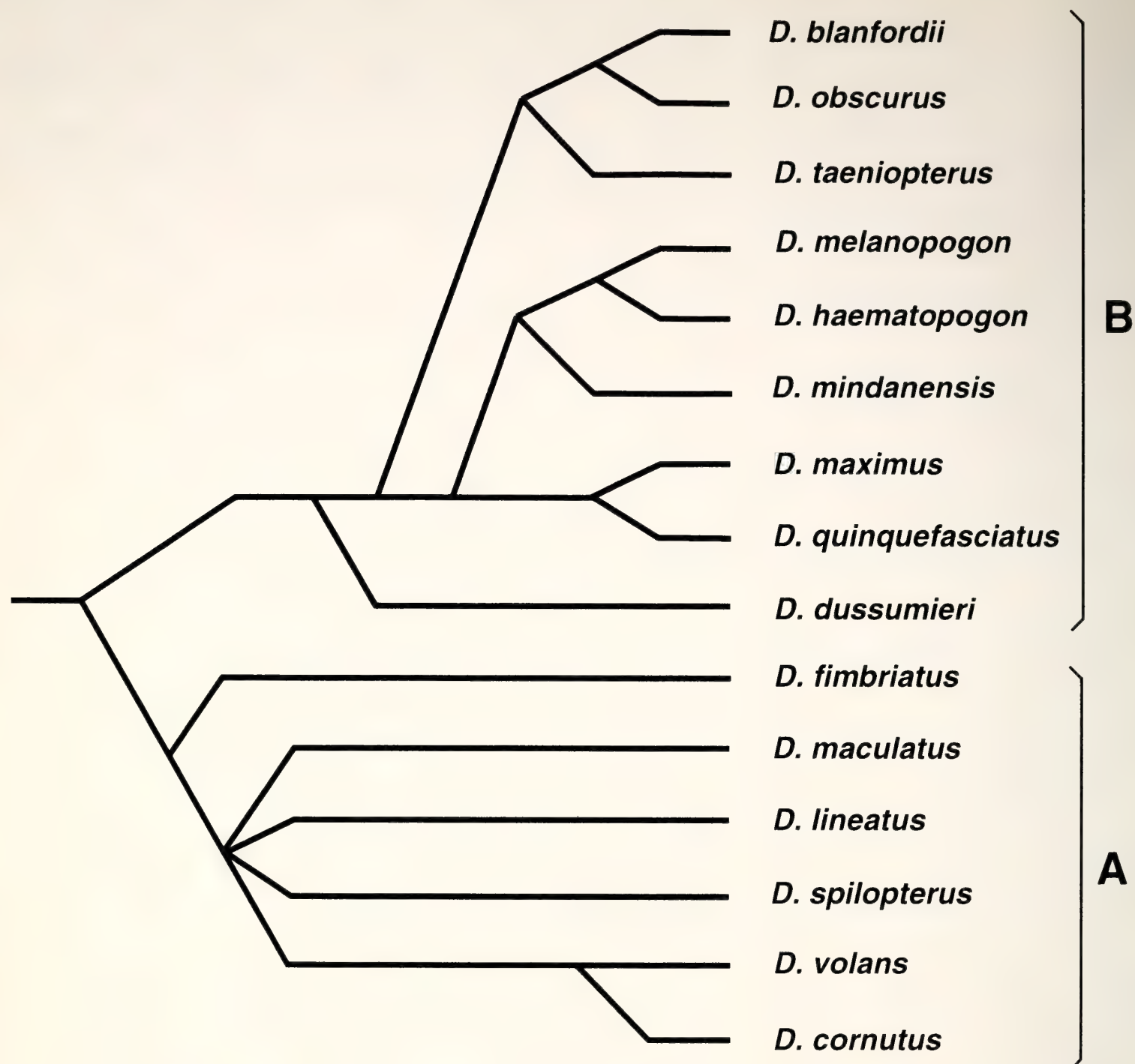


FIG. 1. Phylogenetic tree of the genus *Draco*, proposed by Musters (1983) on the basis of morphological characters and geographic ranges. A: group of species characterized by outward-directed nostrils and more or less developed nuchal crests; B: group of species characterized by upward-directed nostrils and absence of distinct nuchal crests.

to species examined in their studies on the basis of great morphological similarities. The absence of those eight species from the analyses does not thus seem to have imposed any crucial effect on the resultant general picture of the phylogeny and biogeography of the genus (Honda et al., 1999b). In contrast, absence of the other species, *D. fimbriatus*, was supposedly more problematic because its relationship with other congeneric species was uncertain

(Musters, 1983).

In the present study, we sequenced a part of mitochondrial DNA for *D. fimbriatus* and analyzed the sequence data with comparable published data for other congeneric species. Our purposes were to elucidate the phylogenetic position of *D. fimbriatus*, and to discuss the phylogenetic relationships and historical biogeography of the genus accordingly.

MATERIALS AND METHODS

Samples Analyzed

A female *Draco fimbriatus*, collected from Gunung Gate, West Java [Kyoto University Zoological Collection (KUZ) 49655], was examined. We also incorporated into the analyses data published for other *Draco* species, as well as for representatives of several other agamid genera (Honda et al., 1999b, c, 2000) (Table 1). Data for *Bradypodion fischeri* of the Chamaeleonidae and *Iguana iguana* of the Iguanidae, possible closest relatives of the Agamidae and Acrodonta (i.e., an assem-

blage of the Chamaeleonidae and the Agamidae), respectively (Frost and Etheridge, 1989), were also incorporated into the analyses (Ota et al., 1999; Honda et al., 2000).

In some species of *Draco*, morphological differentiations have been reported between conspecific subspecies and/or populations (e.g., Hennig, 1936; Taylor, 1963; Inger, 1983; Musters, 1983). However, conspecific samples were designated as a single operational taxonomic unit (OTU), because our previous study showed that all conspecific samples exclusively constitute lowest clusters with high bootstrap values

TABLE 1. Localities of *Draco* and other agamid samples used for analyses. Data sources are (a) Honda et al. (1999a); (b) Honda et al. (1999b); (c) Honda et al. (2000); (d) Ota et al. (1999); (e) this study. See Appendix for further details.

Sample	Locality
<i>D. blanfordii</i>	Thailand ^b
<i>D. cornutus</i>	Borneo ^a
<i>D. dussumieri</i>	India ^b
<i>D. fimbriatus</i>	Java ^e
<i>D. haematopogon</i>	Peninsular Malaysia ^b
<i>D. lineatus beccarii</i>	Sulawesi ^b
<i>D. maculatus</i>	Thailand ^b
<i>D. maximus</i>	Borneo ^b
<i>D. melanopogon</i>	Thailand ^b
<i>D. obscurus</i>	Borneo ^b
<i>D. quinquefasciatus</i>	Peninsular Malaysia ^b
<i>D. taeniopterus</i>	Thailand ^b
<i>D. volans</i>	Java ^b
<i>Acanthosaura crucigera</i>	Thailand ^c
<i>Agama stelio</i>	West Asia or North Africa ^c
<i>Aphaniotis fusca</i>	Peninsular Malaysia ^b
<i>Calotes versicolor</i>	Thailand ^c
<i>Gonocephalus grandis</i>	Peninsular Malaysia ^c
<i>Japalura polygonata</i>	Japan ^c
<i>Lophognathus temporalis</i>	New Guinea ^c
<i>Phoxophrys nigrilabris</i>	Borneo ^c
<i>Phrynocephalus axillaris</i>	Central Asia ^c
<i>Physignathus cocincinus</i>	Thailand ^c
<i>Ptyctolaemus phuwanensis</i>	Thailand ^b
<i>Bradypodion fischeri</i>	Africa ^c
<i>Iguana iguana</i>	America ^d

(Honda et al., 1999b).

The specific arrangement of *Draco* follows that by Musters (1983) (also see Honda et al., 1999b). As in our previous study (Honda et al., 1999b), we were unable to examine *D. mindanensis*, *D. spilopterus*, and six recently described or revalidated species (Lazell, 1987, 1992; Ross and Lazell, 1990). Of these, *D. biaro*, *D. bimaculatus*, and *D. caerulhians* were assumed to be closest to *D. lineatus*, *D. ornatus* to *D. spilopterus*, and *D. everetti* and *D. jareckii* to *D. volans* on the basis of their great morphological similarities. We believe these designations do not lead to any substantial error in the results of the phylogenetic analyses (Honda et al., 1999b).

Extraction, Amplification and Sequencing of DNA

DNA extraction, amplification and sequencing are described in detail elsewhere (Honda et al., 1999b, c). Approximately 850 base pairs (bp) of the mitochondrial 12S and 16S rRNA genes were amplified using the polymerase chain reaction (PCR; Saiki et al., 1988) with primers L1091 and H1478 (Kocher et al., 1989), and L2606 and H3056 (Hedges et al., 1993), respectively.

Phylogenetic Analyses

Alignments for DNA sequences were determined based on maximum nucleotide similarity using CLUSTAL W 1.4 (Thompson et al., 1994). The neighbor-joining (NJ) method (Saitou and Nei, 1987) was applied to infer relationships among taxa with a pairwise matrix of distance using Kimura's two-parameter model (Kimura, 1980). NJ analysis was performed with PHYLIP 3.54c (Felsenstein, 1993). Degrees of support for internal branches in each tree were assessed by 1,000 bootstrap pseudoreplications (Felsenstein, 1985). For maximum-likelihood (ML) analysis and maximum-parsimony (MP) analysis, fastDNAm1 1.0.6 (Olsen et al., 1994) and PAUP* 4.0b (Swofford, 1998) with the heuristic search

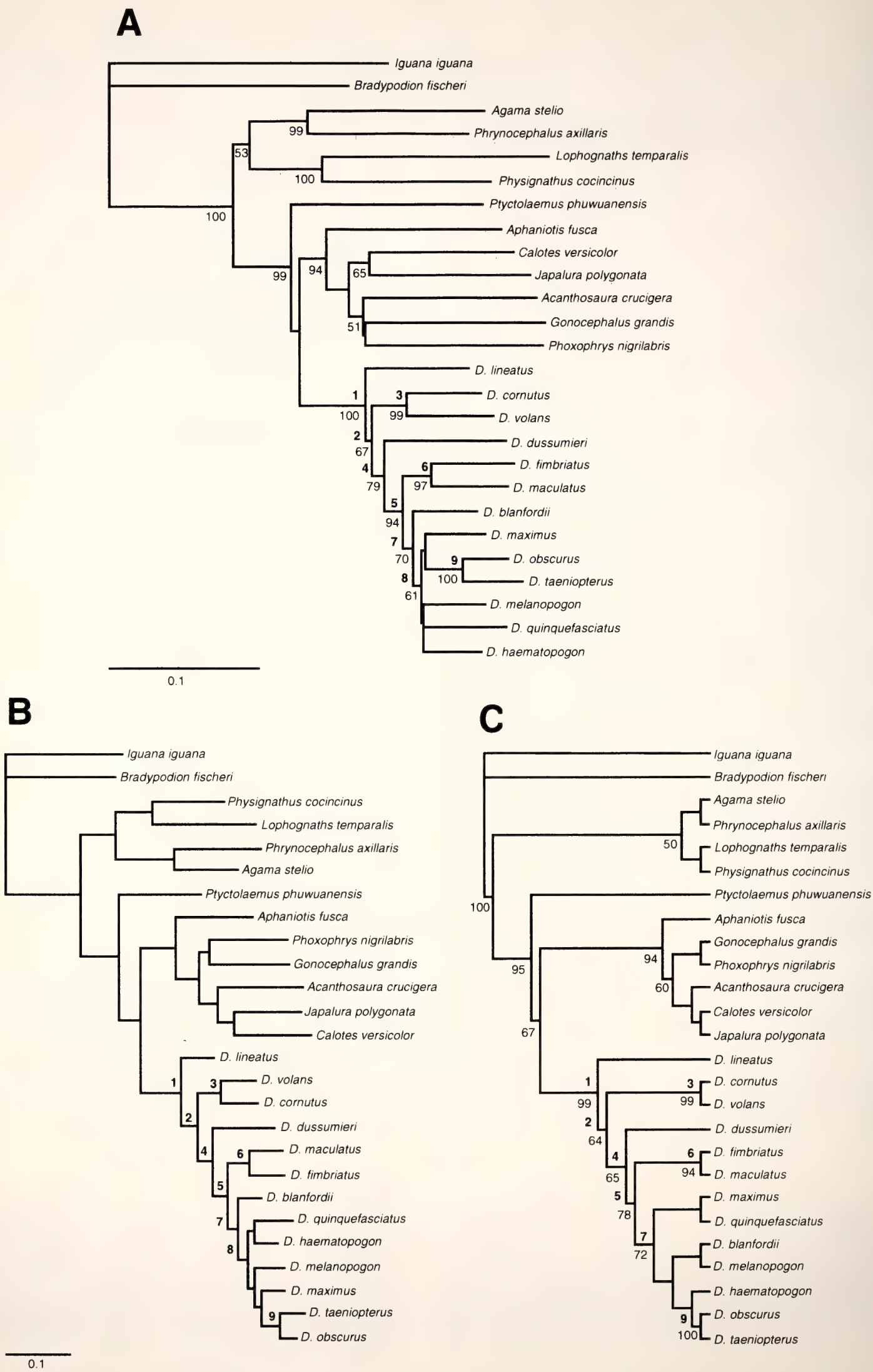
option were used, respectively. Confidence was assessed by 1,000 bootstrap replications. In these three analyses, insertions and deletions were excluded, and transition:transversion bias was assumed as 2:1 according to the observed ratio for the ingroup (transition/transversion = 1.96).

RESULTS

Aligned sequences from two mitochondrial genes (848 bp in total) are presented in Fig. 2. The 12S rRNA fragment consisted of a total of 412 sites, 303 of which were variable. For the 16S rRNA fragment, there was a total of 436 aligned sites, 260 of which were variable. Inter-generic nucleotide replacements within the Agamidae varied from 133 bp (*Calotes* vs. *Japalura*) to 236 bp (*Calotes* vs. *Lophognathus*). Inter-specific nucleotide replacements within *Draco* varied from 48 bp (*D. obscurus* vs. *D. taeniopterus*) to 120 bp (*D. taeniopterus* vs. *D. volans*). Nucleotide replacements between *D. fimbriatus* and other congeneric species were observed from 73 bp (vs. *D. maculatus*) to 116 bp (vs. *D. lineatus*).

The NJ dendrogram derived from aligned sequences is shown in Fig. 3A. Monophyly of *Draco* was supported with complete bootstrap proportions (BPs) (node 1: BP = 100%). Within the *Draco* cluster, we recognized eight nodes (nodes 2–9), as more or less substantial, because these were supported with BPs $\geq 50\%$. *Draco lineatus* diverged first, followed by the *D. cornutus*—*D. volans* cluster (node 3: BP = 99%), *D. dussumieri*, the *D. fimbriatus*—*D. maculatus* cluster (node 6: BP = 97%), and *D. blanfordii* in that order, leaving *D. haematopogon*, *D. maximus*, *D. melanopogon*, *D. obscurus*, *D. quinquefasciatus*, and *D. taeniopterus* as monophyletic (node 8: BP = 61%). Within node 8, *D. obscurus* and *D. taeniopterus* formed an exclusive cluster (node 9: BP = 100%).

The results of ML (Fig. 3B) and MP analyses (Fig. 3C) showed no inconsistency



with the NJ dendrogram in terms of branching topology of nodes 1–9 at the level of BPs $\geq 50\%$ or P-values ≤ 0.01 except for the absence of node 8 in MP.

DISCUSSION

Phylogenetic relationships of Draco

Musters (1983) recognized two major lineages within *Draco* (Fig. 1), that were characterized by outward-directed nostrils and more or less developed nuchal crests (Group A), and upward-directed nostrils and the absence of distinct nuchal crests (Group B). Character states in the former are reasonably assumed to be primitive (Honda et al., 1999b). Musters (1983) postulated the most basal divergence of *D. fimbriatus* in Group A (Fig. 1).

Honda et al. (1999b), on the basis of mitochondrial DNA sequence data, negated this dichotomy because of the presence of four distinct, deeply diverged evolutionary lineages: *D. lineatus*, the *D. cornutus*—*D. volans* clade, *D. dussumieri*, and the clade consisting of *D. blanfordii*, *D. haematopogon*, *D. maculatus*, *D. maximus*, *D. melanopogon*, *D. obscurus*, *D. quinquefasciatus* and *D. taeniopterus* (henceforth referred to as clade α). The relationship resulting from the present analyses by incorporating the DNA sequence data from *D. fimbriatus* also substantially differs from that resulting from the morphological analysis by Musters (1983) in indicating the sister relationship of *D. fimbriatus* with *D. maculatus*. This suggests that *D. fimbriatus* is a member of clade α , and that the

character states found in this species but lacking in most other species including *D. maculatus* (e.g., long head: Musters, 1983) have evolved from states of corresponding characters in *D. maculatus*.

The addition of *D. fimbriatus* to the analysis otherwise yielded results largely similar to those of Honda et al. (1999b), and further confirmed the close affinity between *D. dussumieri* and node 5 (= clade α) among the four major clades. The present results also indicate that *D. lineatus* diverged first, followed by the *D. cornutus*—*D. volans* cluster and *D. dussumieri* in that order, leaving node 5 as monophyletic.

With respect to the relationships of *D. blanfordii*, *D. obscurus* and *D. taeniopterus*, the present results, which point to the non-monophyly and the sister relationship between *D. obscurus* and *D. taeniopterus*, contradict Inger's (1983) and Musters' (1983) views, which assumed the closest affinity of these three species and their relationships to be expressed as (*D. taeniopterus*, (*D. blanfordii*, *D. obscurus*)), respectively. Moreover, our results suggest the early divergence of *D. blanfordii* from the other Southeast Asian species with derived character states (see above). Morphological similarities among these species (nuchal fold, enlarged scales on gular pouch, and five ribs in the patagium: Inger, 1983; Musters, 1983) thus seem to be attributable to convergence rather than to recent common ancestry. The character state of obliquely upward directed nostrils (Inger, 1983, but described as "upward or slightly posteriorly directed" by Musters [1983]) ob-

FIG. 3. (A) NJ dendrogram of 26 taxa derived from a distance matrix of 12S and 16S rRNA sequence data. Numbers beneath branches are bootstrap proportions (BPs) of at least 50% of the 1,000 bootstrap pseudoreplications. Nodes with bold numbers are identical with ML and MP analyses. Bar equals 0.1 unit of Kimura's two-parameter distance. (B) ML dendrogram (ln likelihood = -10,519.6). All branches were supported as significantly positive ($p < 0.01$). Bar equals 0.1 unit. (C) MP cladogram using the heuristic search option (3,015 steps, 409 bp informative under the condition of parsimony, consistency index = 0.408). Branches without BP values were not supported in $\geq 50\%$ of the replicates.

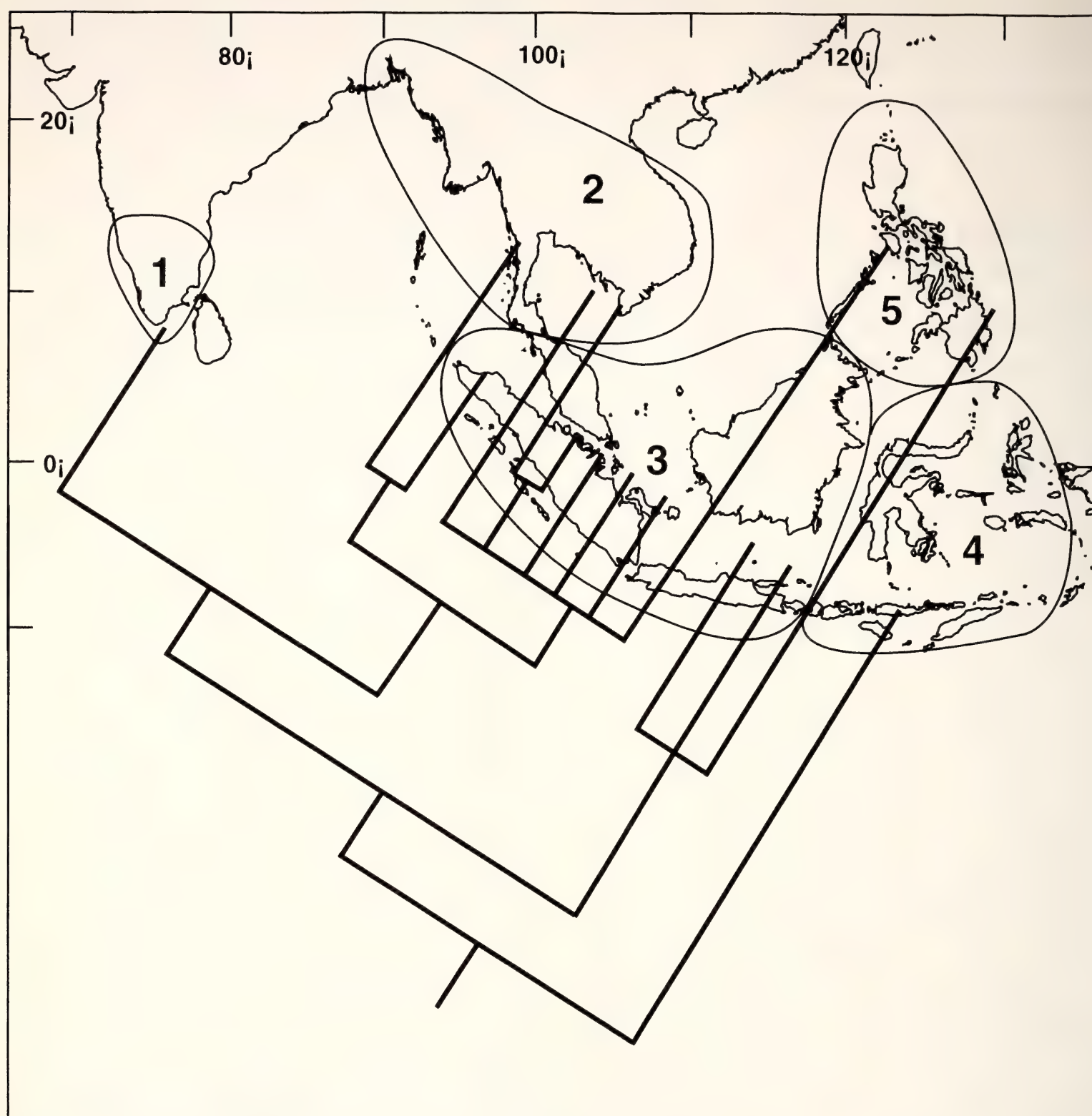


FIG. 4. A map of Southeast and South Asia showing distributions of *Draco* species. Five areas are recognized on the basis of distributional patterns in *Draco* and other taxonomic groups (Wallace 1860; Dunn and Dunn, 1977; Musters, 1983; Heaney, 1984, 1991; Alcalá, 1986; Holloway, 1987; Lazell, 1987, 1992; Ross and Lazell, 1990) following Honda et al. (1999b): 1, southern India; 2, Indochinese Peninsula; 3, Malay Peninsula and the Greater Sunda Islands exclusive of Sulawesi; 4, Lesser Sunda Islands, Sulawesi and Maluku Islands; 5, Philippines. *Draco dussumieri* is the only species distributed in Area 1, *D. blanfordii*, *D. maculatus*, and *D. taeniopterus* in Area 2, *D. cornutus*, *D. fimbriatus*, *D. haematopogon*, *D. maximus*, *D. melanopogon*, *D. obscurus*, and *D. quinquefasciatus* in Area 3, *D. lineatus* in Area 4, and *D. mindanensis* and *D. spilopterus* in Area 5. Of these, *D. blanfordii*, *D. maculatus*, and *D. taeniopterus* also occur in the northern part of Area 3, whereas *D. obscurus* is distributed in the southern part of Area 2 as well across the boundary in the Malay Peninsula. *Draco lineatus*, while mainly occurring in Area 4, also occurs in the southeastern part of Area 3 and the southern part of Area 5 as well. *Draco volans* has the widest distribution which, while seemingly centering in Area 3, also partially encompasses Areas 2, 4 and 5.

served in *D. blanfordii*, may represent an intermediate state between the two extreme states of this character (outward and upward directions: see above).

Biogeography of *Draco*

Figure 4 depicts the phylogeographical hypothesis of *Draco* resulting from the present analyses. Honda et al. (1999b) divided the geographic range of *Draco* into five areas (as numbered in Fig. 4), and constructed the zoogeographical scenario consisting of five stages as follows: (i) The presumptive ancestor of *Draco* first possibly diverged into three groups, the ancestors of *D. lineatus*, the *D. volans*—*cornutus* cluster, and the cluster consisting of *D. dussumieri* and clade α (see above) through a series of vicariations among Areas 4, 3, and 2; (ii) the common ancestor of *D. dussumieri* and clade α invaded Area 1 from Area 2, and there (area 1) it was isolated and diverged into *D. dussumieri*; (iii) the common ancestor of clade α invaded Area 3 from Area 2, and there (Area 3) the Southeast Asian members of Group B diverged from the *maculatus*-like ancestor; (iv) the resultant ancestors of *D. blanfordii* dispersed into Area 2 from Area 3; and (v) invasion of Area 2 by the ancestor of *D. taeniopterus* from Area 3 followed (iv). Of these, however, the reliability of stages (i), (ii), and (iii) were not supported with sufficient BPs in the DNA sequence or allozyme analysis by Honda et al. (1999b).

The present results support these five hypothetical stages with significant BPs or P-values, enabling us to extend the scenario for the historical biogeography of *Draco* as follows. (I) The primary divergence of *Draco* occurred between *D. lineatus* in Area 4 and the other (node 2) occurred in Areas 2 and 3. (II) The common ancestor of the latter (node 2) further split into the *D. volans*—*cornutus* clade (node 3) in Area 3 and the other (node 4) split in Area 2. (III) The ancestor of *D. dussumieri* diverged in Area 1 from the common ancestor of node

4, leaving node 5 (=clade α : Honda et al., 1999b) as monophyletic. (IV) The common ancestor of node 5 (=clade α), originally distributed in Area 2, dispersed into Area 3, and then it split into the *D. fimbriatus*—*maculatus* clade (node 6) and the Southeast Asian members of Group B (node 7) in Areas 2 and 3, respectively. (V) The ancestor of node 7 further diverged into several species in Area 3. (VI) The ancestors of *D. fimbriatus* independently dispersed from Area 2 into Area 3. (VII) The ancestors of *D. blanfordii* invaded Area 2 from Area 3. (VIII) The ancestor of *D. taeniopterus* independently dispersed from Area 3 into Area 2. Hypothetical stages (VII) and (VIII) are consistent with stages (iv) and (v) of Honda et al. (1999b), respectively. These two stages seem to be supported by the early divergence of *D. blanfordii* from node 8, and by the sister relationship between *D. taeniopterus* and *D. obscurus* (node 9) in the present study. This idea is also circumstantially supported by the fact that *D. blanfordii* has diverged into several subspecies within Area 2, whereas *D. taeniopterus* is rather monomorphic (Musters, 1983), and that *D. blanfordii* dispersed into the northern and eastern parts of Area 2, whereas *D. taeniopterus* is confined to the southern part of this region (Taylor, 1963; Musters, 1983).

Two endemic species, *D. mindanensis* and *D. spilopterus*, are distributed in Area 5. Diversity in Area 5 is assumed to have increased through multiple colonizations from outside (*D. mindanensis* from Area 3, and *D. spilopterus* from Area 3 or Area 4), not through an endemic radiation (Honda et al., 1999b). In Area 4 where the range of *D. lineatus* is centered, *D. volans* also occurs. Occurrence of *D. volans* in this area seems to have resulted from a relatively recent dispersal from Area 3. The taxonomic diversity of *Draco* in Areas 2 and 3 is also considered to have increased through multiple colonizations. We suspect that the diversity of this genus in each area of the

Southeast Asian region has increased chiefly through multiple colonizations rather than through *in situ* diversifications.

ACKNOWLEDGMENTS

We would like to thank M. Kobayashi for laboratory assistance and K. Araya for providing specimens of *D. fimbriatus*. We are also much indebted to N. Murakami for useful suggestions on phylogenetic analyses. Special thanks are due to N. Satoh and members of his laboratory for continuous support for our laboratory experiments. Experiments were also carried out using the facilities of the Kyoto University Museum. Our research was partially supported by the Nakayama Foundation for Human Science.

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APPENDIX

Accession Numbers of Specimens.—The DDBJ accession numbers of 12S and 16S rRNAs are presented.

Acanthosaura crucigera: AB031963, AB031980. *Agama stelio*: AB031976, AB031993. *Aphaniotis fusca*: AB023749, AB023771. *Calotes versicolor*: AB031964, AB031981. *Draco blanfordii blanfordii*: AB023733, AB023751. *D. cornutus*: AB023728, AB023752. *D. dussumieri*: AB023734, AB023753. *D. haematopogon*: AB023735, AB023754. *D. lineatus beccarii*: AB023737, AB023756. *D. maculatus maculatus*: AB023739, AB023758. *D. maximus*: AB023740, AB023760. *D. melanopogon*: AB023741, AB023761. *D. melanopogon*: AB023742, AB023762. *D. obscurus obscurus*: AB023743, AB023763. *D. quinquefasciatus*: AB023745, AB023765. *D. taeniopterus*: AB023747, AB023767. *D. volans volans*: AB023748, AB023770. *Gonocephalus grandis*: AB031966, AB031983. *Japalura polygonata polygonata*: AB031968, AB031985. *Phoxophrys nigrilabris*: AB031971, AB031988. *Phrynocephalus axillaris*: AB031972, AB031989. *Physignathus cocincinus*: AB031973, AB031990. *Ptyctolaemus phuwuanensis*: AB023750, AB023772. *Bradypodion fischeri*: AB031962, AB031979. *Iguana iguana*: AB028742, AB028756.

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***Mabuya cumingi* (Reptilia: Scincidae): An Addition to the Herpetofauna of Lanyu Island, Taiwan**

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Abstract: An adult male *Mabuya*, recently collected from Lanyu Island, Taiwan, was identified as *Mabuya cumingi*, a species hitherto known only from Luzon Island of the Philippines, on the basis of small body size (56.6 mm in snout-vent length), embossed dorsal head scales, five scales beneath toe I, and the presence of a dark middorsal stripe. Occurrence of this species on Lanyu Island was also confirmed by additional sighting records. *Mabuya cumingi* is regarded as a fourth reptile species representing a dispersal to this island from the Philippines.

Key words: *Mabuya cumingi*; *Mabuya multicarinata*; Scincidae; Lanyu Island; Taiwan; Philippines

INTRODUCTION

Lanyu Island is an islet of 45.7 km² in area and 548 m in height, and is located ca. 60 km southeast of the main island of Taiwan and ca. 390 km north of Luzon Island, the Philippines. Since the description of *Gekko kikuchii* by Oshima (1912), a total of 18 species of reptiles, including three possible human commensals (*Hemidactylus frenatus*, *Hemiphyllodactylus typus typus*, and *Ramphotyphlops braminus*) and 15 putative native species, have been recorded from this island (Ota, 1991a,b; Lue et al., 1999).

A number of previous biogeographers ar-

gued that the fauna of Lanyu Island is characterized by a higher proportion of Philippine elements when compared with that of the main island of Taiwan (e.g., Kuroda, 1925; Kano, 1933, 1936). Of the 15 putative native reptiles hitherto known from Lanyu Island, three (*G. kikuchii*, *Lepidodactylus yami*, and *Mabuya multicarinata borealis*) are classified as the Philippine elements, whereas most of the remaining species are considered to represent dispersals from the main island of Taiwan (Ota, 1991a,b).

Recently, one of us (WH) collected a specimen of a strange skink from Lanyu Island. This specimen, deposited in the herpetological collection of the National Museum of Natural Science, Taichung, as NMNS 3371, had keeled scales on the dorsum of the body and completely separated pterygoids with a palatal notch extending

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forwards to the level of the center of the eye, and thus was identified as a member of the genus *Mabuya* (see Greer [1970, 1977]). In this paper, we describe external characters of this specimen in detail, and compare it with species of *Mabuya* known from Taiwan and the Philippines to determine its specific status.

MATERIAL AND METHODS

The specimen was collected by hand from sparse grass near the coast, 3 km north of Tung-Ching Village, Lanyu Island (22°04'N, 121°33'E), on 17 July 1999. After being photographed in life (Fig. 1), the lizard was fixed in 10% formalin, preserved in 70% ethanol, and then subjected to detailed examination of morphological characters. Measurements were taken to the nearest 0.1 mm with dial calipers. Definitions of characters follow those of Brown and Alcalá (1980).

RESULTS

Description of the present specimen

A male with well-developed testes and

convoluted epididymides; snout-vent length (SVL) 56.6 mm; tail length 99.4 mm; head length 12.2 mm; head width 8.1 mm; snout-eye length 4.7 mm; eye length 3.5 mm; ear length 1.1 mm; snout-forelimb distance 20.8 mm; axilla-groin distance 28.8 mm; forelimb length 17.7 mm; hindlimb length 25.8 mm.

Snout largely tapered, but rounded at tip; scales on dorsal surface of head embossed; rostral approximately twice as broad as high, rounded dorsally, in contact with frontonasal; supranasals long, narrow, not in contact at midline; frontonasal nearly as broad as frontal, in contact with frontal; prefrontals separated at midline; frontal elongate, broadly in contact with second supraoculars; supraoculars four, first smallest, second largest; frontoparietals not fused; interparietal incomplete, fused to parietals posteriorly; nuchals in two pairs; postnasal absent; anterior loreal distinctly higher than long, much shorter than posterior loreal; supralabials seven, fifth largest, beneath eye; seven infralabials; ear small, without lobules; each scale on dorsal and lateral surfaces of body with six or seven keels, ventral scales smooth, or slight-



FIG. 1. Dorsolateral view of the specimen of *Mabuya cumingi* from Lanyu Island, Taiwan (NMNS 3371: SVL = 56.6 mm).

ly rugose; 30 rows of scales around mid-body; 43 vertebral scale rows between parietals and base of tail just above vent; preanals slightly enlarged; limbs well developed; finger III almost as long as finger IV; toe IV distinctly longer than toe III; subdigital scales 5-5, 9-9, 14-14, 15-15 and -8 on left-right fingers I, II, III, IV and V (left finger V damaged), and 5-5, 10-10, 15-16, 21-21 and 13-13 on left-right toes I, II, III, IV and V, respectively; tail not damaged or regenerated.

In life, dorsal surfaces of head and body light grayish tan, with black middorsal stripe; additional longitudinal black stripes, separated from each other by white interspaces, in dorsolateral, lateral, and ventrolateral regions; lateral stripe, running from nasal and eye to above base of hindlimb broad, the others narrower (Fig. 1). In preservative, dorsal ground color and white interspaces faded to dark gray and light gray, respectively, making longitudinal dark stripes indistinct.

Comparisons

Three species of *Mabuya*, *M. longicaudata*, *M. multifasciata*, and *M. multicarinata borealis*, have been recorded from Taiwan (Ota et al., 1994; Lue et al., 1999). The present specimen differs from the three spe-

cies in a much smaller body size, because adult SVLs of these species well exceed 60 mm (Okada et al., 1992; Ota et al., 1994). Furthermore, it differs from *M. longicaudata* and *M. multifasciata* in having distinctly embossed head scales, because in the latter species, scales covering the dorsal surface of head are smooth, or only slightly rugose (Ota et al., 1994). From *M. multicarinata borealis*, the present specimen also differs in having a greater number of vertebral scale rows (43, vs. 34-41), fewer scales beneath toe IV (21, vs. 24-28), and a distinct middorsal stripe (vs. no middorsal stripe: Ota, 1991a).

Most of those and other characteristics of NMNS 3371 are shared with *M. cumingi* and *M. indepressa* from the Philippines (Brown and Alcala, 1980). So, we compare the present specimen with these two species in detail (Table 1). Brown and Alcala (1980) reported that both of these Philippine species have a distinct interparietal which almost always separates parietals completely. In the present specimen, however, the interparietal is incomplete because it is fused with parietals posteriorly. The Lanyu specimen differs from *M. indepressa* in having shorter hindlimbs in relation to the axilla-groin distance, and fewer scales beneath toe I. States of these and

TABLE 1. Comparisons of characters among the Lanyu specimen of *Mabuya*, and *M. cumingi* and *M. indepressa* from the Philippines. Abbreviations are: SVL, snout-vent length; HLL, hindlimb length, AGD, axilla-groin distance; IP, interparietal; MSR, midbody scale rows; VS, vertebral scale rows; TIS, subdigital scales beneath the toe I; TIVS, subdigital scales beneath the toe IV.

Character	Lanyu specimen	<i>M. cumingi</i> *	<i>M. indepressa</i> *
SVL (mm)	56.6	39.5-54.0	45.6-66.6
HLL/AGD (%)	90.0	74-96	94-116
HLL/SVL (%)	45.6	37-45	45-58
IP	incomplete	distinct	distinct
MSR	30	28-32	30-34
VS	43	40-47	41-48
TIS	5	5-6	6-8
TIVS	21	16-21	18-24

* Data for these species in the Philippines were taken from Brown and Alcala (1980).

most other characters in the present specimen are shared with *M. cumingi*, except for SVL, which is slightly greater in the Lanyu specimen than in the latter.

DISCUSSION

Morphological comparisons indicate that the Lanyu specimen most resembles *M. cumingi*, but differs from the species in having slightly greater SVL and an incomplete interparietal. Considering that the body size often varies extensively among conspecific insular populations in reptiles (see Ota et al [1995] and papers referred therein for examples), such a difference in SVL between the present specimen and *M. cumingi* from the Philippines may have little taxonomic significance. Moreover, it is probable that the interparietal condition in the Lanyu specimen represents an anomaly rather than a stable population feature. We thus identify the specimen as *M. cumingi*. Further detailed analysis of variation on the basis of additional specimens is desired to verify those assumptions.

During the fieldwork on Lanyu Island in 1995 (by HO) and 1997–1999 (by WH), both of us observed several other individuals with body size and color pattern similar to those of the present specimen. Thus, although the present record was made only on the basis of a single voucher specimen, we are almost certain that there is an established breeding population of *M. cumingi* on Lanyu Island. All individuals sighted were found in relatively open environments near the coast, and appeared to be most active on clear mornings and at dusk.

In the synopsis of the lizards of Taiwan, Lin and Cheng (1990) provided three color photographs as those of *M. multicarinata*. The animals photographed, however, showed a color pattern almost identical with that of the present specimen including a distinct middorsal stripe on body. Therefore, although the locality of the individual photographed was not given in that book, it is

most likely that it actually represented *M. cumingi* from Lanyu Island rather than *M. multicarinata*.

Since the original description by Brown and Alcala (1980), *Mabuya cumingi* has been known only from Luzon Island. Thus, the present finding seems to represent another example of reptilian dispersals to Lanyu Island from the Philippines (Ota, 1991a, b).

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Sperm Morphology of Some Indian Frogs as Revealed by SEM

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Abstract: The size and shape of 15 species of frogs (Ranidae and Rhacophoridae) from southwestern India were investigated by light and scanning electron microscopy. Most species of the genus *Rana* had spermatozoa of a typical form, with a thick sperm head and a thin tail. In contrast, *Rana beddomii* had long spermatozoa with a slender and densely coiled sperm head and a thick tail, suggesting the validity of the genus *Indirana*. The sperm head of *Nyctibatrachus major* was thick and very loosely coiled. Differing from *Rhacophorus* species from east Asia, the sperm head of *Rh. malabaricus* was not coiled. *Polypedates maculatus* had very long thread-like spermatozoa as in *Rh. malabaricus*. Spermatozoa of all examined species of the genus *Philautus* had a crescent-like sperm head and a thin tail resembling the head and tail of the genus *Chirixalus*.

Key words: Anura; Spermatozoa; SEM; India

INTRODUCTION

To date, spermatozoa of more than a hundred species of frogs have been investigated by light microscopy (LM), SEM or TEM (e.g., Lee and Jamieson, 1992; Kuramoto, 1998; van der Horst et al., 1995). From these studies, it became evident that sperm morphology is very variable between taxa and useful for elucidating taxonomic relationships between them. For example, Kuramoto (1996) showed that four rhacophorid genera from Japan and Taiwan have each a distinctive form of spermatozoa and, within the genus *Buergeria*, the form of the spermatozoon of *B. buergeri* is apparently derived from the

sperm form of its congeners. It was also suggested that sperm morphology reflects the mode of reproduction (van der Horst et al., 1995).

India has a rich amphibian fauna, involving more than 180 species (Inger and Dutta, 1986). Among these, only two species, *Bufo stomaticus* and *Rana tigerina*, have been studied spermatologically (Sharma and Dhindsa, 1955; Sharma and Sekhri, 1955). The primary purpose of these studies was to clarify the process of spermatogenesis and thus detailed descriptions of sperm are not given.

We examined sperm morphology of several frog species from Karnataka and Kerala, southwestern India by light microscopy (LM) and scanning electron microscopy (SEM). Dubois (1992) revived several subfamilies and genera in the Rani-

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dae and his revision has induced much confusion and debate on their validity. In our present materials representatives of some of his genera are involved, and we expect that our spermatological work will provide cues to resolve this taxonomic problem. Also, we examine generic differentiation in the Rhacophoridae including the genera *Philautus* for which sperm morphology has not yet been reported.

MATERIALS AND METHODS

Frogs used in this study were: *Rana temporalis* from Madikeri, Karnataka; *R. malabarica* from Cannanore, Kerala; *R. limnocharis* from Madikeri; *R. keralensis* from Madikeri; *R. syhadrensis* from Mangalore, Karnataka; *R. cyanophlyctis* from Mangalore; *R. tigerina* from Mangalore; *R. beddomii* from Madikeri; *Nyctibatrachus major* from Madikeri (Ranidae); *Rhacophorus malabaricus* from Madikeri; *Polypedates maculatus* from Madikeri; *Philautus* sp. A from Mangalore; *Philautus* sp. B from Mangalore; *Philautus* sp. C from Kudremukh, Karnataka; and *Philautus* sp. D from Kudremukh (Rhacophoridae). All specimens were collected in June and July 1999.

Philautus species, small arboreal frogs, are difficult to identify as Inger and Stuebing (1997, p. 163) admitted, and our specimens did not seem to fit the descriptions of *Philautus* species from the Western Ghats (Ahl, 1931; Daniels, 1998; Inger et al., 1984; Rao, 1937). Brief descriptions of our specimens are given here: *Philautus* sp. A was reddish brown on the back, some with irregular dark blotches, underside immaculate, tympanum about half of eye diameter and lower half of tympanum white, snout rather pointed, ca. 24 mm in SVL; *Philautus* sp. B was pale gray on the back with an inverted irregularly U-shaped blackish mark, underside with many scattered small dark markings, tympanum small and indistinct, snout not pointed, ca. 25 mm in

SVL; *Philautus* sp. C was dark brown on the back with a large hourglass-like pale marking from eye to vent, underside vermiculate with brown, yellow, and white, two distinct large yellowish spots on anterior surface of thigh, snout not pointed, ca. 18 mm in SVL; *Philautus* sp. D was pale yellow to reddish yellow on the back and underside including the vocal sac, some with three dark indistinct longitudinal stripes on the back, tympanum small and indistinct, ca. 26 mm in SVL.

We collected several other unidentified species in the genera *Rana* and *Philautus*, and we will refer to sperm morphology of these species briefly.

The testis was squashed in a small quantity of water with forceps, and the sperm suspension thus prepared was put on slides and on cover slips, fixed with 2% glutaraldehyde for about one hour, and air-dried. Spermatozoa on the slide were stained with Giemsa for LM and those on cover slips were coated with gold and observed with a scanning electron microscope JSM-T200 (JEOL). Two males were used for each species.

RESULTS

Excepting *Rana beddomii*, all seven species of the genus *Rana* had very similar spermatozoa with a thick sperm head and a thin tail (Fig. 1). Sperm sizes were also similar, ranging from about 15 to 20 μm in head length and from about 45 to 70 μm in total length, but variable in head width (Table 1). The acrosome and the middle piece or neck piece were discernible externally from the head proper by their slightly narrower width. The tail was about 0.2 μm in width. Three unidentified species from Mangalore and Kudremukh, apparently belonging to the *Rana limnocharis* complex, had spermatozoa which were similar to those of the above species.

Spermatozoa of *R. beddomii* were very long, about twice as long as those of the

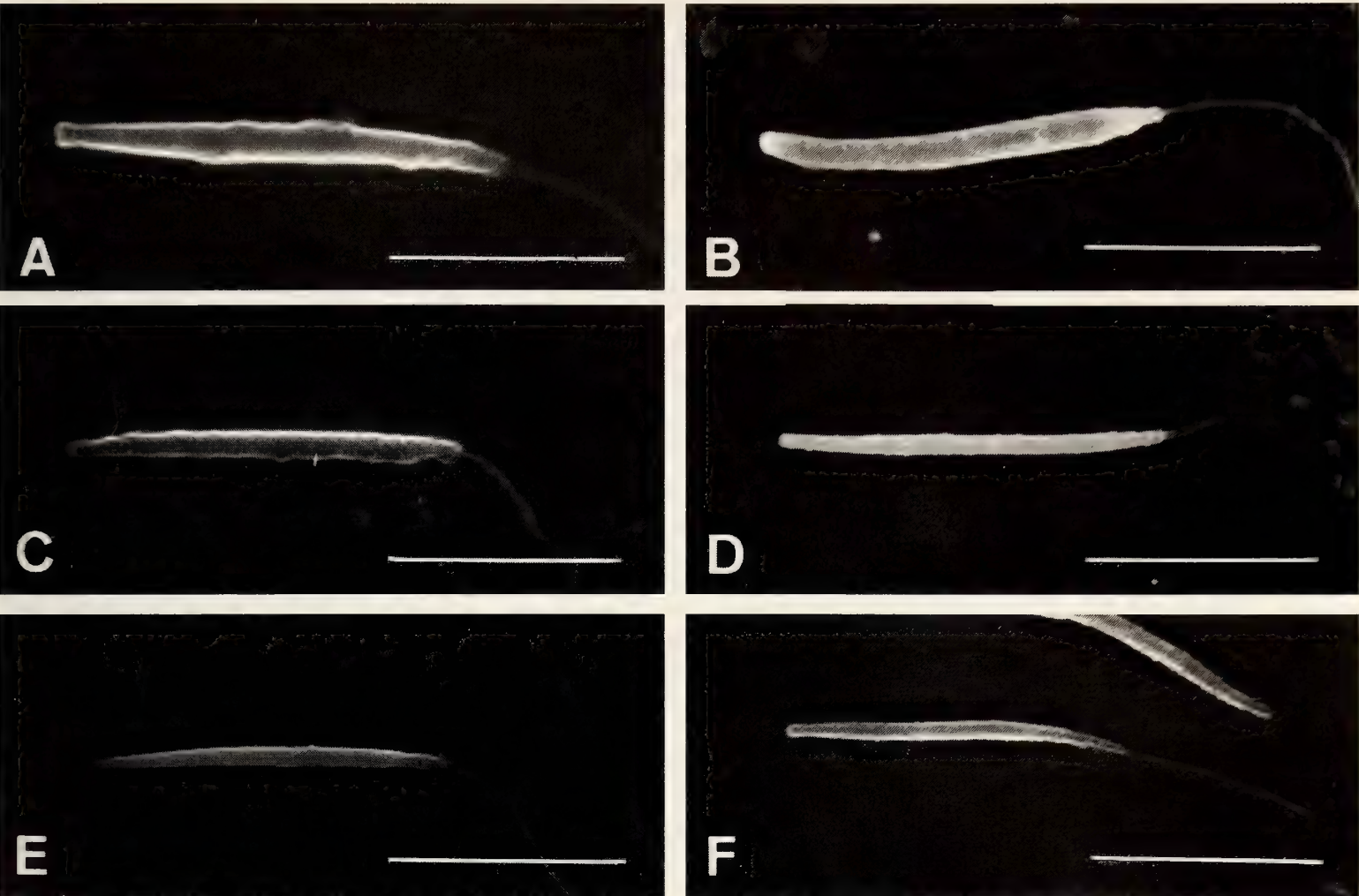


FIG. 1. Spermatozoa of *Rana temporalis* (A), *R. malabarica* (B), *R. limnocharis* (C), *R. keralensis* (D), *R. syhadrensis* (E), *R. cyanophlyctis* (F), and *R. tigerina* (G). Scales equal 10 μ m.

other species of the genus *Rana* (Fig. 2, Table 1). The sperm head formed an elon-

gated, densely and sinistrally coiled spiral, about 0.5 μ m in diameter of spiral and

TABLE 1. Sperm sizes in 15 species of frogs from southwestern India (Mean \pm SD in μ m, N=10).

	Head length	Tail length	Total length	Head width
<i>Rana temporalis</i>	20.3 \pm 0.82	45.4 \pm 3.26	65.5 \pm 3.23	1.7 \pm 0.10
<i>Rana malabarica</i>	17.2 \pm 1.49	27.9 \pm 2.78	45.0 \pm 2.81	1.6 \pm 0.17
<i>Rana limnocharis</i>	17.4 \pm 1.28	38.2 \pm 3.13	55.6 \pm 3.38	1.2 \pm 0.09
<i>Rana keralensis</i>	16.5 \pm 0.94	40.4 \pm 4.25	56.9 \pm 4.67	1.1 \pm 0.06
<i>Rana syhadrensis</i>	18.3 \pm 2.90	36.7 \pm 7.11	55.0 \pm 9.16	0.9 \pm 0.07
<i>Rana cyanophlyctis</i>	17.0 \pm 2.05	54.2 \pm 3.78	71.2 \pm 4.57	0.9 \pm 0.05
<i>Rana tigerina</i>	15.0 \pm 1.43	47.1 \pm 2.99	62.1 \pm 3.67	0.9 \pm 0.08
<i>Rana beddomii</i>	37.5 \pm 0.90	79.2 \pm 5.99	116.7 \pm 6.10	0.5 \pm 0.03
<i>Nyctibatrachus major</i>	23.8 \pm 3.12	40.1 \pm 7.11	63.9 \pm 7.28	0.8 \pm 0.06
<i>Rhacophorus malabaricus</i>	52.3 \pm 2.44	92.6 \pm 6.93	144.9 \pm 5.96	0.6 \pm 0.05
<i>Polypedates maculatus</i>	76.5 \pm 4.20	84.0 \pm 14.1	160.5 \pm 14.6	0.4 \pm 0.05
<i>Philautus</i> sp. A	21.6 \pm 2.76	28.4 \pm 4.74	50.0 \pm 5.21	0.8 \pm 0.05
<i>Philautus</i> sp. B	23.8 \pm 2.77	28.5 \pm 2.66	52.3 \pm 4.38	0.8 \pm 0.04
<i>Philautus</i> sp. C	19.0 \pm 2.28	30.9 \pm 4.88	49.9 \pm 4.56	0.8 \pm 0.03
<i>Philautus</i> sp. D	20.6 \pm 3.39	29.7 \pm 3.50	50.3 \pm 3.63	0.8 \pm 0.05

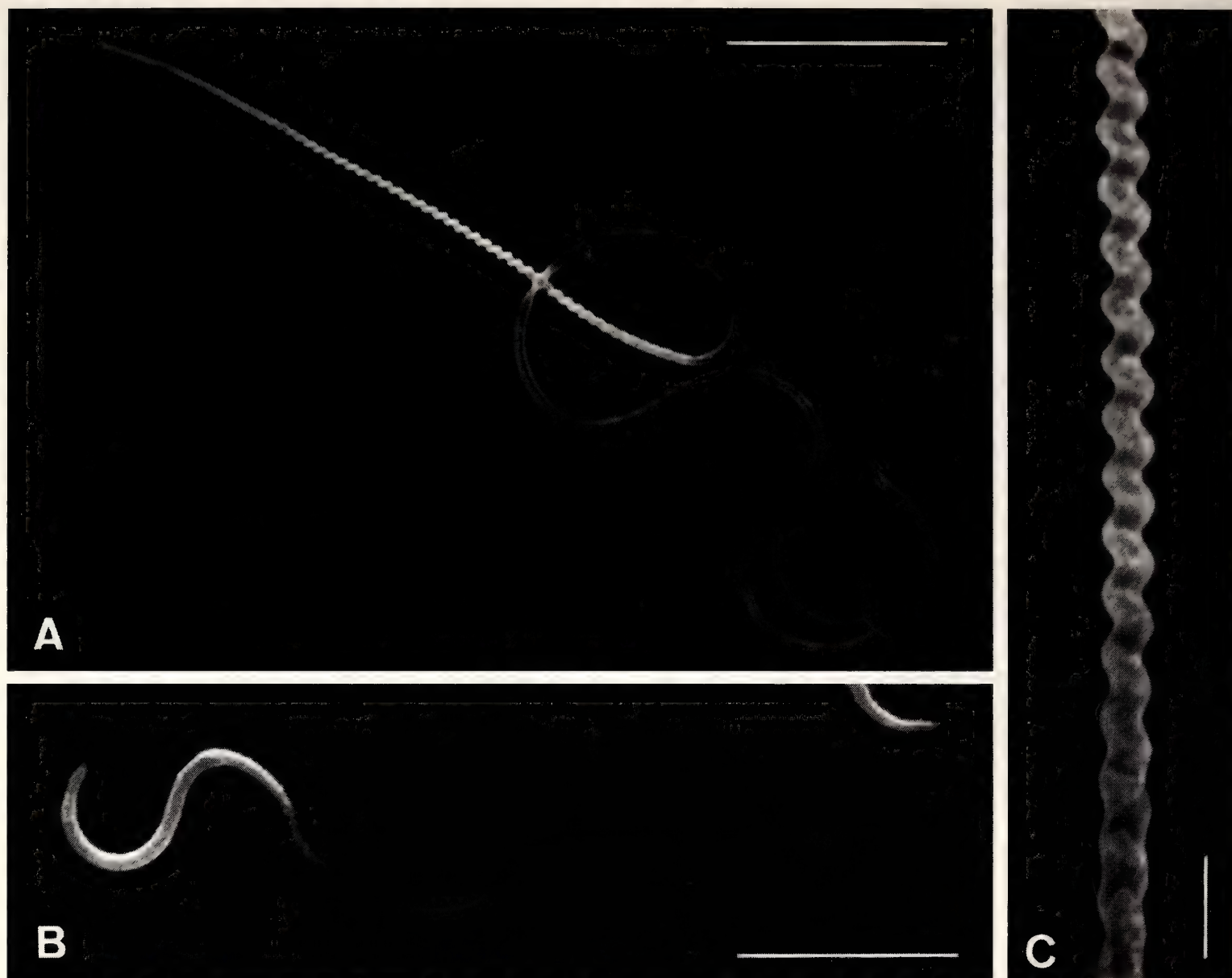


FIG. 2. Spermatozoa of *Rana beddomii* (A), *Nyctibatrachus major* (B), and a part of the coiled sperm head of *R. beddomii* (C). The lower part of the coil in (C) is the basal portion of the head. Scales equal 10 μm in (A) and (B), and 1 μm in (C).

about 0.3 μm in width of coil fiber. "Head length" of *R. beddomii* in Table 1 shows the length of the coil, not the net length of coil fiber. The tail width (about 0.5 μm) was nearly the same as head width and much wider than those of its congeners. This type of spermatozoa is new to our knowledge of anuran sperm morphology. We examined the sperm of a frog from Madikeri which was similar to *R. beddomii* but could not be precisely identified. This frog had spermatozoa which were nearly identical to those of *R. beddomii*. The testis of *R. beddomii* and the unidentified *beddomii*-like frog was considerably larger than that of the other ranid frogs.

Nyctibatrachus major had also peculiar

spermatozoa (Fig. 2). Typically the sperm head was S-shaped, undoubtedly forming a loose coil three-dimensionally. A slightly thin portion at the tip of head may be the acrosome and that at the end may be the middle piece. The tail was 0.13 μm in width, much thinner than most species of the genus *Rana*. Total sperm length was within the range of most *Rana* species. This type of spermatozoa has not been reported in Ranidae. The testis was much smaller than in most ranid species.

Rhacophorus malabaricus had long thread-like spermatozoa, and *Polypedates maculatus* had still longer ones (Fig. 3, Table 1). The acrosome and the middle piece could not be distinguished externally in

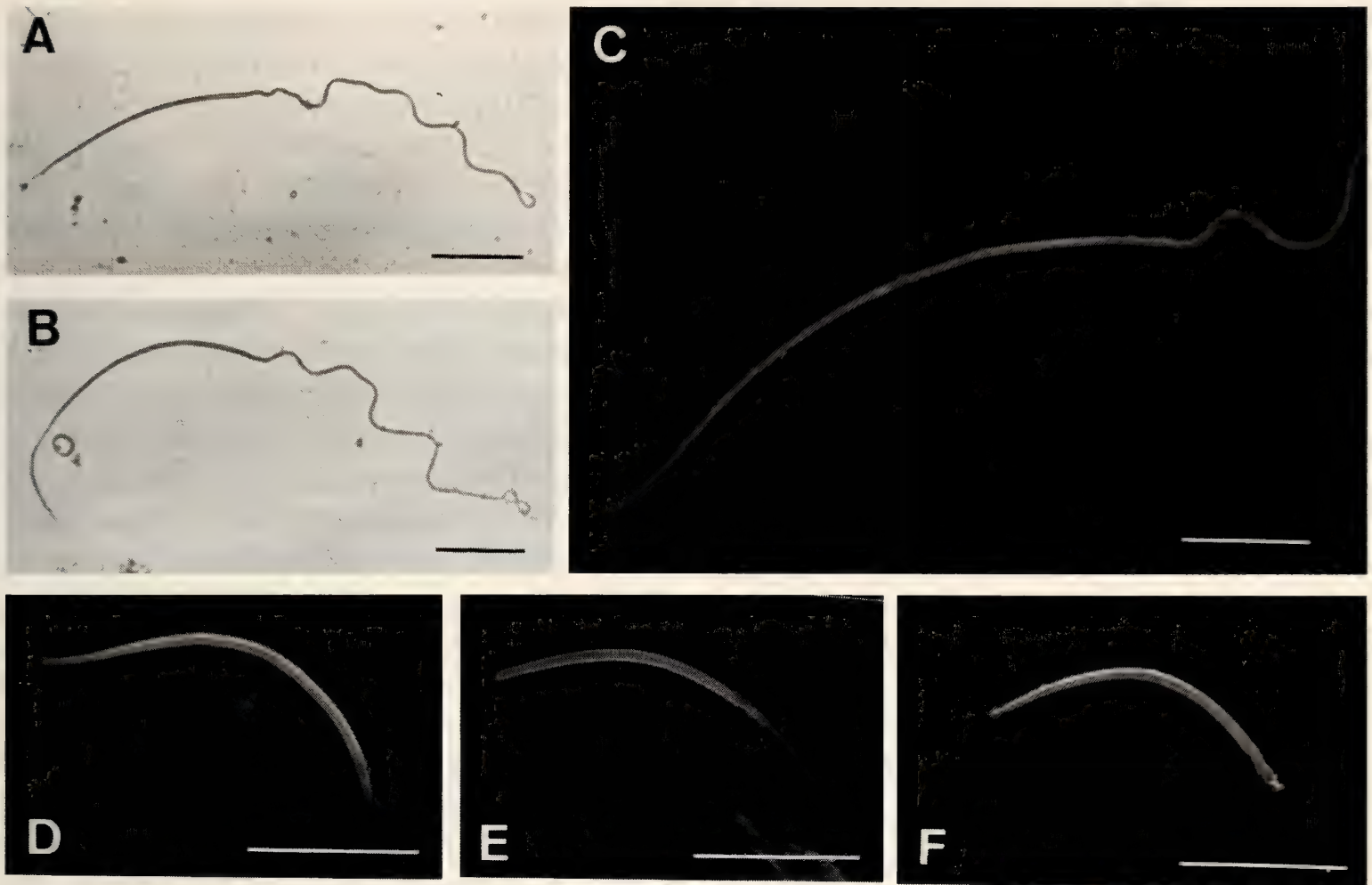


FIG. 3. Spermatozoa of *Rhacophorus malabaricus* (A, C), *Polypedates maculatus* (B), *Philautus* sp. A (D), *Philautus* sp. B (E), and *Philautus* sp. D (F). (A) and (C) were taken by LM. Scales equal 20 μm in (A) and (B), and 10 μm in (C)–(F).

either species. The tail was thick, only slightly thinner than the sperm head with a width of about 0.4 to 0.5 μm .

The four species of the genus *Philautus* had spermatozoa of nearly the same size as those in the genus *Rana* (Table 1). The sperm head was invariably bent slightly, forming a crescent (Fig. 3). The middle piece was granular in appearance. The tail was thin, about 0.2 μm in width. We examined sperm of three other species of *Philautus* (unidentified) and all were very similar to that of the above four species in every characteristic.

DISCUSSION

Sperm shape and size of the seven species of the genus *Rana* examined in this study (*temporalis*, *malabarica*, *limnocharis*, *keralensis*, *syhadrensis*, *cyanophlyctis*, *tigeri-*

na) are essentially identical to those of the other *Rana* species reported so far, in that all have a thick sperm head and a thin tail (Kuramoto, 1998; van der Horst et al., 1995). The sperm form of *R. tigerina* agrees well with the description by Sharma and Sekhri (1955). The sperm head of *R. limnocharis* is much longer than that of the same species from Japan and China (Kuramoto, 1998), suggesting intraspecific geographic variations in sperm size as exemplified in Japanese salamanders (Kuramoto, 1997). Noticeable exceptions in the genus *Rana* were three species of the *Rana narina* complex and *R. ishikawae* which had very long, slender spermatozoa (Kuramoto, 1998).

Rana beddomii had a completely different type of sperm which was unlike any other sperm type found in anurans. The sperm head is long and densely coiled, and the tail

is thick suggesting involvement of filamentous components other than a single flagellum. Detailed TEM analysis is needed to clarify this unusual feature of sperm. In the *Rana* species here examined, *limnocharis*, *keralensis*, and *syhadrensis* are frequently allocated to the genus *Limnonectes*, *cyanophlyctis* to the genus *Euphlyctis*, *tigerina* to the genus *Hoplobatrachus* (as *H. tigerinus*), and *beddomii* to the genus *Indirana* (Duellman, 1993; Dutta and Manamendra-Arachchi, 1996). The genera *Limnonectes*, *Euphlyctis*, and *Hoplobatrachus* do not show any sign of sperm differentiation as already pointed out by Kuramoto (1998) in *Limnonectes* and *Hoplobatrachus*, whereas *Indirana* differs completely from other genera in sperm type, indicating a different phyletic lineage. The karyotype of *R. beddomii* ($2n=24$, Joshy et al., 1999) also differs from the other Indian ranid frogs. These suggest strongly the validity of the genus *Indirana*. It should be confirmed whether the other species which are allocated to *Indirana* have the same sperm type.

The sperm head of *Nyctibatrachus major* was very loosely coiled. The sperm shape of *Xenopus laevis* (Bernardini et al., 1986, 1988; Yoshizaki, 1987) resembles that of *N. major*. Some pelobatid frogs, *Megophrys montana* (Asa and Phillips, 1988) and four species of *Vibrissaphora* (Wu and Yang, 1981) have spermatozoa which are coiled several times. According to Asa and Phillips (1988), chromatin condenses to form a cylindrical coil within a spherical nucleus of spermatids without participation of microtubular manchette. The occurrence of this sperm type in several unrelated anuran families suggests a common cytological basis for shaping this loosely coiled sperm head. The coil structure of the sperm head found in *R. beddomii* and *Rhacophorus* species inhabiting east Asia may also have the same basis. All types of coiled sperm head reported so far are coiled sinistrally, and this also suggests a common underlying

mechanism for producing coiled nuclear condensation.

The sperm size of *Rh. malabaricus* is within the range of east Asiatic *Rhacophorus* species (Kuramoto, 1996). Contrary to the compactly coiled sperm heads of these other species, however, the sperm head of *Rh. malabaricus* is straight. We could not observe even a trace of coiled structure at all. It is necessary to examine the sperm shapes of southeast Asiatic species to clarify sperm variations within this genus. Sperm of *Polypedates maculatus* is nearly the same as those of east Asiatic *Polypedates* species in both shape and size.

The crescent-like sperm heads of the genus *Philautus* are very similar to those of *Chirixalus* species from east Asia (Kuramoto, 1996), suggesting a close relationship of the two genera. The relationship from spermatological data fits the phylogeny of Channing (1989) fairly well, but not that of Liem (1970). This shape of sperm head may link straight and loosely coiled types.

Van der Horst et al. (1995) suggested a correlation between sperm size and the mode of fertilization in South African frogs, that is, terrestrial fertilizers have long spermatozoa and aquatic fertilizers short ones. Kuramoto (1998), examining his spermatological data, doubted this tendency. In the present materials, we observed that a *R. beddomii*-like frog lays eggs in seepages often exposed directly to the air and the eggs were enveloped with tough gelatinous coats. This mode of reproduction is not purely aquatic, so the longer sperm of *R. beddomii* seems to fit the tendency of van der Horst et al. (1995). However, sperm of *R. tagoi* of Japan which has a similar mode of reproduction and the same kind of gelatinous envelope does not differ from that of the other aquatic fertilizers (Kuramoto, 1998). The terrestrial reproductive mode of *Rhacophorus* and *Polypedates* seems to agree with the tendency, but that of *Philautus* seems to contradict it. Although direct development in *Philau-*

tus was reported in species of Philippines (Alcala and Brown, 1982) and Malaysia (Yong et al., 1988) and not in Indian species, Inger et al. (1984) collected most of their *Philautus* specimens from places far from any stream or pond. We also collected calling male specimens in forests or bushes usually far from water. These suggest a terrestrial mode of reproduction, but sperm of *Philautus* are short. Rao (1937) described larvae of some *Philautus* species from streams of Kempholey, Hassan, Karnataka. All have a flattened body, a long muscular tail with a low tail fin, and large mouth parts; all are characteristics of stream-dwelling larvae. No information about reproduction of Indian *Philautus* are available, however. The presence of free-living aquatic tadpoles does not necessarily mean aquatic egg laying. The sperm size does not seem to relate directly to the mode of reproduction but seems to reflect phylogenetic relationships fairly well.

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On the Monophyly of the Agamid Genus *Gonocephalus* Kaup, 1825 (Reptilia: Squamata): A Chromosomal Perspective

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Abstract: We karyotyped five species of the agamid genus *Gonocephalus*, *G. chamaeleontinus*, *G. liogaster*, *G. bellii*, *G. grandis* (from Peninsular Malaysia), and *G. robinsonii*. Of these, karyotypes of the first four species had several chromosomal characteristics exclusively shared with the previously reported karyotypes of *G. miotympanum* and *G. grandis* (from Borneo), such as the diploid chromosome number (42) and the presence of 22 biarmed macrochromosomes. This seems to support the monophyly of those four species and *G. miotympanum*, probably along with some other species of the genus not yet karyotyped. This hypothesis is premised on our finding of distinct chromosomal characteristics that are indicative of highly derived states in the agamid karyotypes. The karyotype of *G. robinsonii*, while remarkably different from other congeneric karyotypes in exhibiting much smaller diploid (32) and biarmed macrochromosome numbers (12), share these and other chromosomal characteristics with several Australian species. It seems unlikely for the karyotype of *G. robinsonii* to directly emerge from other congeneric karyotypes or *vice versa*. We conclude that the inclusion of this species in *Gonocephalus* would render the genus paraphyletic.

Key words: Reptilia; Agamidae; *Gonocephalus bellii*; *G. chamaeleontinus*; *G. liogaster*; *G. grandis*; *G. robinsonii*; Karyotype; Monophyly

INTRODUCTION

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The agamid genus *Gonocephalus* Kaup, 1825, is a group of moderate-sized to large lizards. Darlington (1957) argued that the

genus is zoogeographically exceptional, because it was then considered to occur on both sides of Wallace's Line, a zoogeographic border between the Oriental and Australian faunas. Based on the microchromosome numbers, Witten (1983) also postulated that the Australian species assigned to *Gonocephalus* at that date represent recent dispersals from Southeast Asia across Wallace's Line.

In his unpublished Ph.D. dissertation, Moody (1980), on the basis of phylogenetic analysis of morphological characters, asserted that the *Gonocephalus* species east of Wallace's line were derived from radiations of the Australian stock, and that they are phylogenetically distant from the Southeast Asian species. He further argued that the genus *Hypsilurus* Peters, 1867, once synonymized to *Gonocephalus* by Boulenger (1885), should be resurrected to accommodate species from the Australian Region. Results of more recent immunogenetic (Baverstock and Donnellan, 1990; King, 1990), karyological (Ota et al., 1992), electron-microscopic (Ananjeva and Matveyeva-Dujsebayaeva, 1996), and molecular studies (Honda et al., 2000) favored Moody's (1980) view. All recent authors, with the exception of a few who have obviously overlooked these works (e.g., Urban, 1999), restrict the application of the generic name, *Gonocephalus*, to the Southeast Asian species (Welch et al., 1990; Manthey and Grossmann, 1997).

In all those works addressing the phylogeny of *Gonocephalus* (sensu lato), however, the species assemblage on the western side of Wallace's Line (i.e., *Gonocephalus* [sensu stricto]), though constituting no less than 16 species (Welch et al., 1990; Manthey and Grossmann, 1997), was represented by very few species. For example, Moody (1980) examined osteological specimens for only five species. Furthermore, Baverstock and Donnellan (1990), Ota et al. (1992), Ananjeva and Matveyeva-Dujsebayaeva (1996), and Honda

et al. (2000) examined only one, two, four, and one species, respectively. Considering that a thorough definition of *Gonocephalus* (sensu stricto) depends only on a few external characters (Moody, 1980; Manthey and Grossmann, 1997), the monophyly of the genus is obviously yet to be examined.

Ota et al. (1992) reported that *G. grandis* and *G. miotympanum*, both from Borneo, share characteristic chromosomal arrangements that are obviously in highly derived states. This suggests that the karyological approach may be an effective way to examine the monophyly of the genus. Therefore, in this study, we karyotyped four additional species of *Gonocephalus* including its type species, *G. chamaeleontinus*, as well as *G. grandis* from the Peninsular Malaysia.

MATERIALS AND METHODS

Except for two male *Gonocephalus robinsonii*, all lizards, collected from Peninsular Malaysia and Pulau Tioman (Table 1), were transported to the laboratory where they were injected intraperitoneally with 0.1 ml of colchicine solution (2 mg/ml) per gram of body weight. Sixteen to 18 h after injection, they were anesthetized with diethyl ether and were dissected to remove femur bones. Bone marrow were flushed out from the bones with Hanks balanced buffer solution. For each sample, the cell suspension was left to stand for 10 min before it was centrifuged at 2000 rpm for 5 min. Bone marrow cells were then treated with hypotonic KCl solution (0.06 mole/l) at room temperature (26–28°C) for 1 h, followed by fixation in a 1 : 3 glacial acetic acid : absolute methyl alcohol mixture. Mitotic chromosome slides were prepared by the splash technique, air-dried, and were stained in 6% Gurr Giemsa (BDH) solution. Mitotic cell slides for the remaining two male *G. robinsonii* were prepared in the field following Ota (1989a), and were stained in 2% Giemsa solution.

TABLE 1. Localities, sizes, and sexual compositions of samples of five *Gonocephalus* species examined in this study.

Species	N			Locality
	Males	Females	Total	
<i>G. bellii</i>	2	3	5	Gombak Forest Reserve, Peninsular Malaysia (03°09' N, 101°39' E)
<i>G. chamaeleontinus</i>	3	0	3	Pulau Tioman, near Peninsular Malaysia (02°49' N, 104°09' E)
<i>G. liogaster</i>	0	3	3	Gombak Forest Reserve, Peninsular Malaysia (03°09' N, 101°39' E)
<i>G. grandis</i>	3	3	6	Pulau Tioman, near Peninsular Malaysia (02°49' N, 104°09' E)
<i>G. robinsonii</i>	3	0	3	Cameron Highlands Peninsular Malaysia (04°28' N, 101°20' E)

Karyotypes were determined for each individual lizard on the basis of 8–20 well-spread metaphase cells. Selected cell spreads were photographed with a Nikon Optiphot 2 Photomicrography camera using Kodak TMAX ASA 100 film. Individual chromosome pairs were arranged in decreasing size. For the calculation of arm ratio for each chromosome pair, the lengths of chromosome arms were measured with a CALCOM digitizer. Terminology for chromosomal descriptions follows Green and Sessions (1991), and the karyotype formula follows Peccinini-Seale (1981). Voucher specimens were deposited in the Zoological Reference Collection, Depart-

ment of Biological Sciences, National University of Singapore (ZRC) and Herpetological Collection, Department of Zoology, Kyoto University (KUZ).

RESULTS

In all *Gonocephalus* species examined, karyotypes consisted of chromosomes forming large and smaller size-groups that are referred to here as macrochromosomes and microchromosomes, respectively (Table 2). Of these, macrochromosomes were all bi-armed, whereas detailed morphology remained undetermined for most microchromosomes. No sex chromosome hetero-

TABLE 2. Karyotypes of species of the genus *Gonocephalus*. M=macrochromomes; m= microchromosomes.

Species	2n	Arm nos. in macro-chromosomes	Chromosomal formula	Source
<i>G. bellii</i>	42	44	22 M + 20 m	this study
<i>G. chamaeleontinus</i>	42	44	22 M + 20 m	this study
<i>G. liogaster</i>	42	44	22 M + 20 m	this study
<i>G. robinsonii</i>	32	24	12 M + 20 m	this study
<i>G. grandis</i> (Pulau Tioman)	42	44	22 M + 20 m	this study
<i>G. grandis</i> (Borneo)	42	44	22 M + 20 m	Ota et al. (1992)
<i>G. miotympanum</i>	42	44	22 M + 20 m	Ota et al. (1992)

morphisms or secondary constrictions were evident in any karyotypes.

Karyotypes of *G. chamaeleontinus*, *G. liogaster*, *G. bellii*, and *G. grandis* consisted of $2n=42$ chromosomes, including 22 macrochromosomes (pairs 1–11) and 20 microchromosomes (pairs 12–21). The macrochromosomes of *G. chamaeleontinus* and *G. liogaster* were all metacentric (Fig. 1). From these, macrochromosomes of *G. bellii* and *G. grandis* differed in including submetacentric elements in pairs 1, 4 and 10, and pairs 2, 5, 7 and 9, respectively (Fig. 2). Thus, the arm numbers in macrochromosomes were 44 in all of the four karyotypes. With respect to the microchromosomes, the largest pair (i.e., pair 12) of the *G. bellii* karyotype was distinctly enlarged compared to the chromosome pair immediately following, thus obscuring the size-gap difference between the macro- and microchromosomes. In contrast, chromosome pair 12 was almost as small as pair 13 in karyotypes of *G. chamaeleontinus*, *G. liogaster*, and *G. grandis*, resulting in a more prominent size-gap difference between the two groups of chromosomes.

The karyotype of *G. robinsonii* differs remarkably from those of the other four *Gonocephalus* species in having substantially fewer ($2n=32$) chromosomes. Of the diploid chromosomes, 12 (pairs 1–6) were metacentric macrochromosomes, whereas the remaining 20 (pairs 7–16) were microchromosomes (Fig. 3). Therefore, the arm number in macrochromosomes of this karyotype equaled 24.

DISCUSSION

Karyotypes of *G. chamaeleontinus*, *G. bellii*, *G. liogaster*, and *G. grandis* from Peninsular Malaysia and Pulau Tioman share similar chromosomal features with those of *G. miotympanum* and *G. grandis* from Borneo (Ota et al., 1992). In contrast, the karyotype of *G. robinsonii* differs strikingly from other congeneric karyo-

types.

In the family Agamidae, two karyomorphs are typical: (1) $2n=34$ or 36 chromosomes, including six pairs of metacentric or submetacentric macrochromosomes and 11 or 12 pairs of microchromosomes; and (2) $2n=46$ or 48 chromosomes, all telocentric chromosomes without a distinct size-break (Bickham, 1984; King, 1981; Moody and Hutterer, 1978; Olmo, 1986; Ota and Hikida, 1989; Solleder and Schmid, 1988; Witten, 1983). One of these karyomorphs is considered to be derived from the other through a series of Robertsonian rearrangements of macrochromosomes, sometimes accompanied with addition or deletion of one microchromosome pair (Bickham, 1984; King, 1981). Judging from the fact that both of the two karyomorphs sometimes occur in a single genus or closely related genera (Gorman and Shochat, 1972; Ota, 1988, 1989b) and that there are so few karyotypes representing intermediate states between the two extremes, such chromosomal rearrangements may proceed rapidly when they are once triggered (King, 1981).

It is obvious that the karyotypes of *G. chamaeleontinus*, *G. bellii*, *G. liogaster*, *G. grandis*, and *G. miotympanum* could not be derived from either of the two typical agamid karyomorphs merely through Robertsonian rearrangements of macrochromosomes and slight numerical changes of microchromosomes, because these karyotypes include a much larger number of banded macrochromosomes (22) than those of any other agamid karyotypes hitherto reported, and at the same time, exhibit a greater diploid number (42) than the agamid karyomorph (1). Moreover, their fundamental number (NF, >64) is considerably greater than those with karyomorph (2) (NF=46 or 48). We thus consider karyotypes of the five *Gonocephalus* species to represent a highly derived state, and that the chromosomal features exclusively shared among these species support

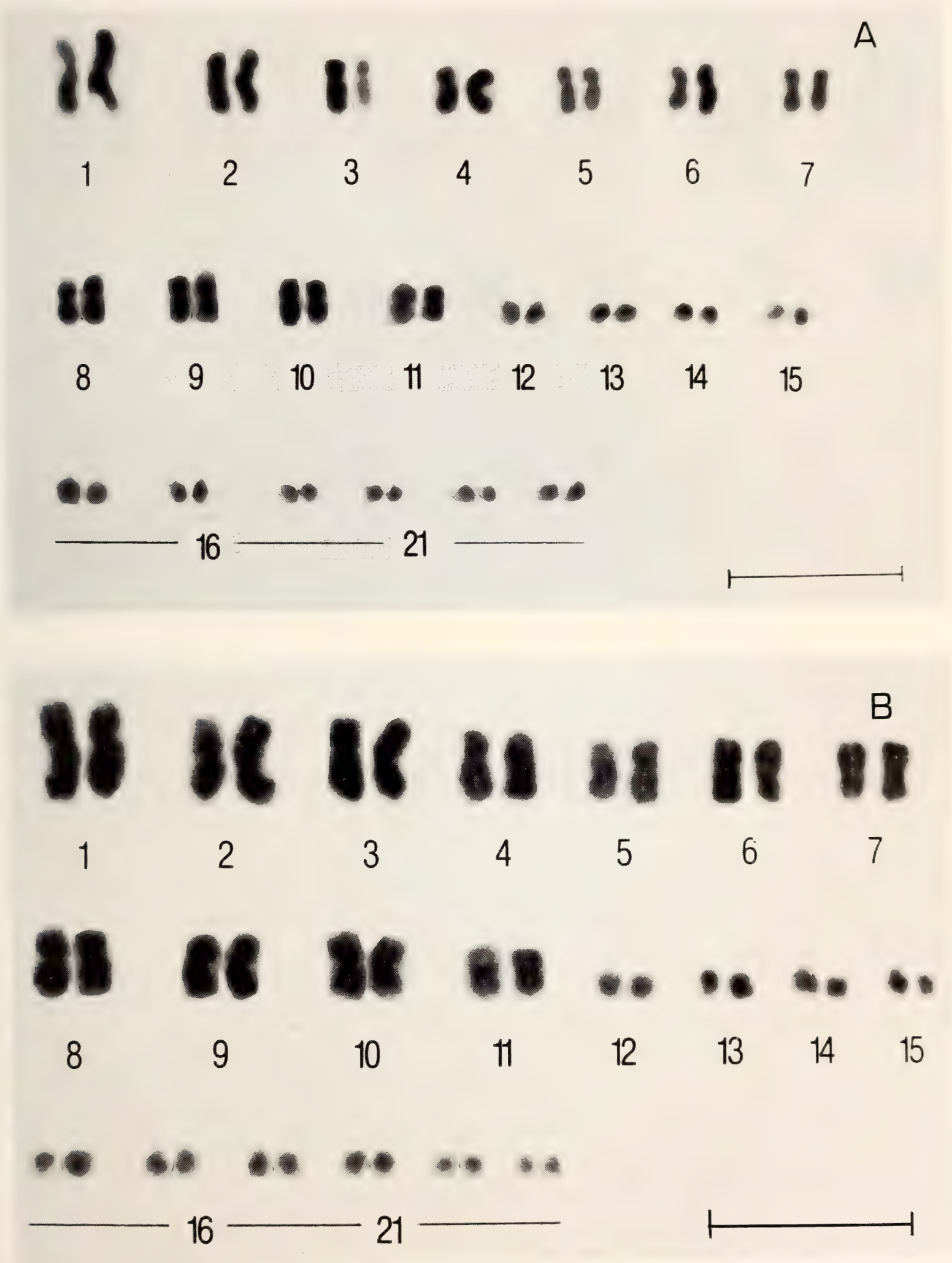


FIG. 1. Karyotypes of (A) *Gonocephalus chamaeleontinus*, and (B) *G. liogaster*. Bars equal 5 μ m.

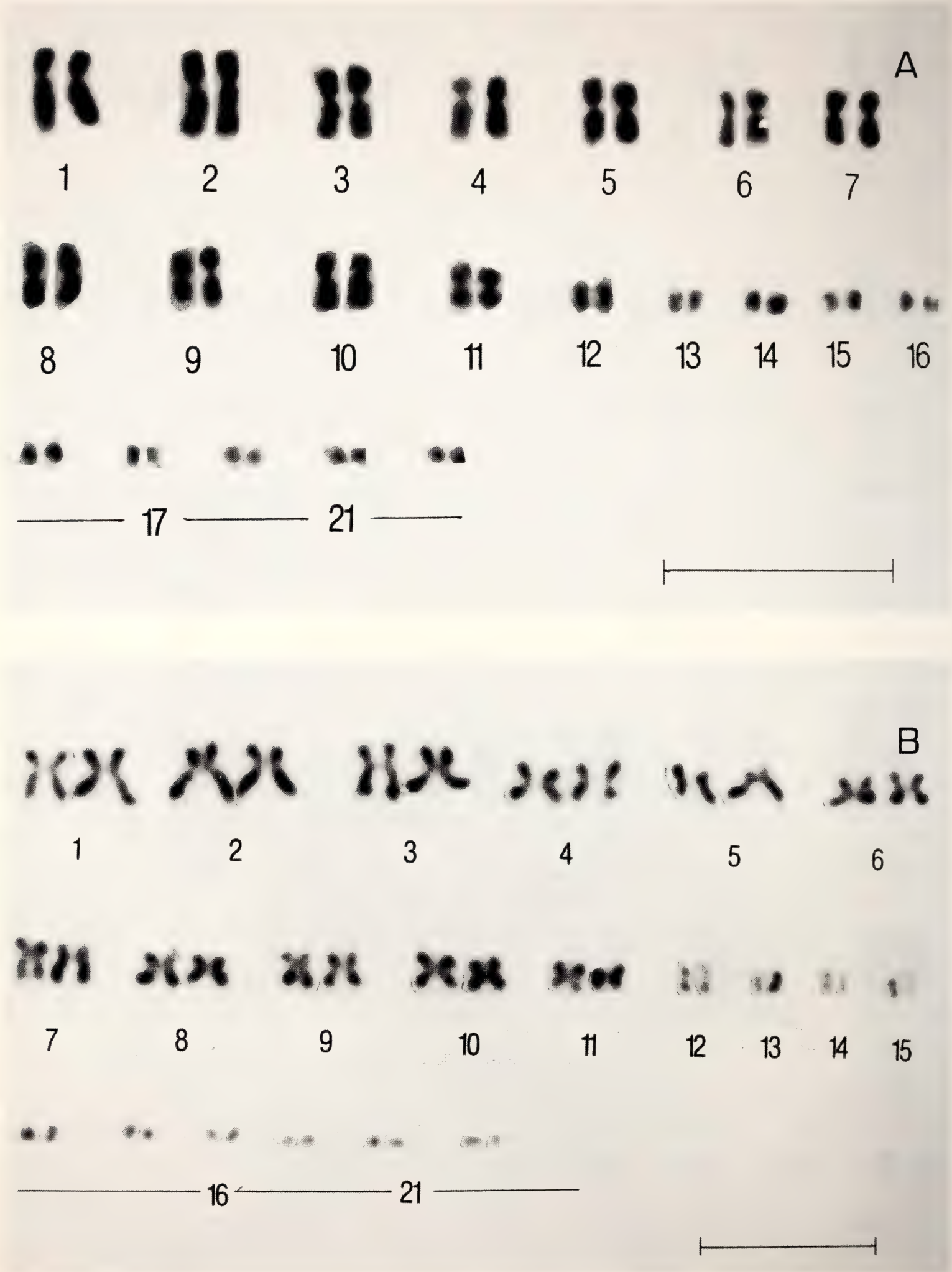


FIG. 2. Karyotypes of (A) *Gonocephalus bellii*, and (B) *G. grandis*. Bars equal 5 μm.

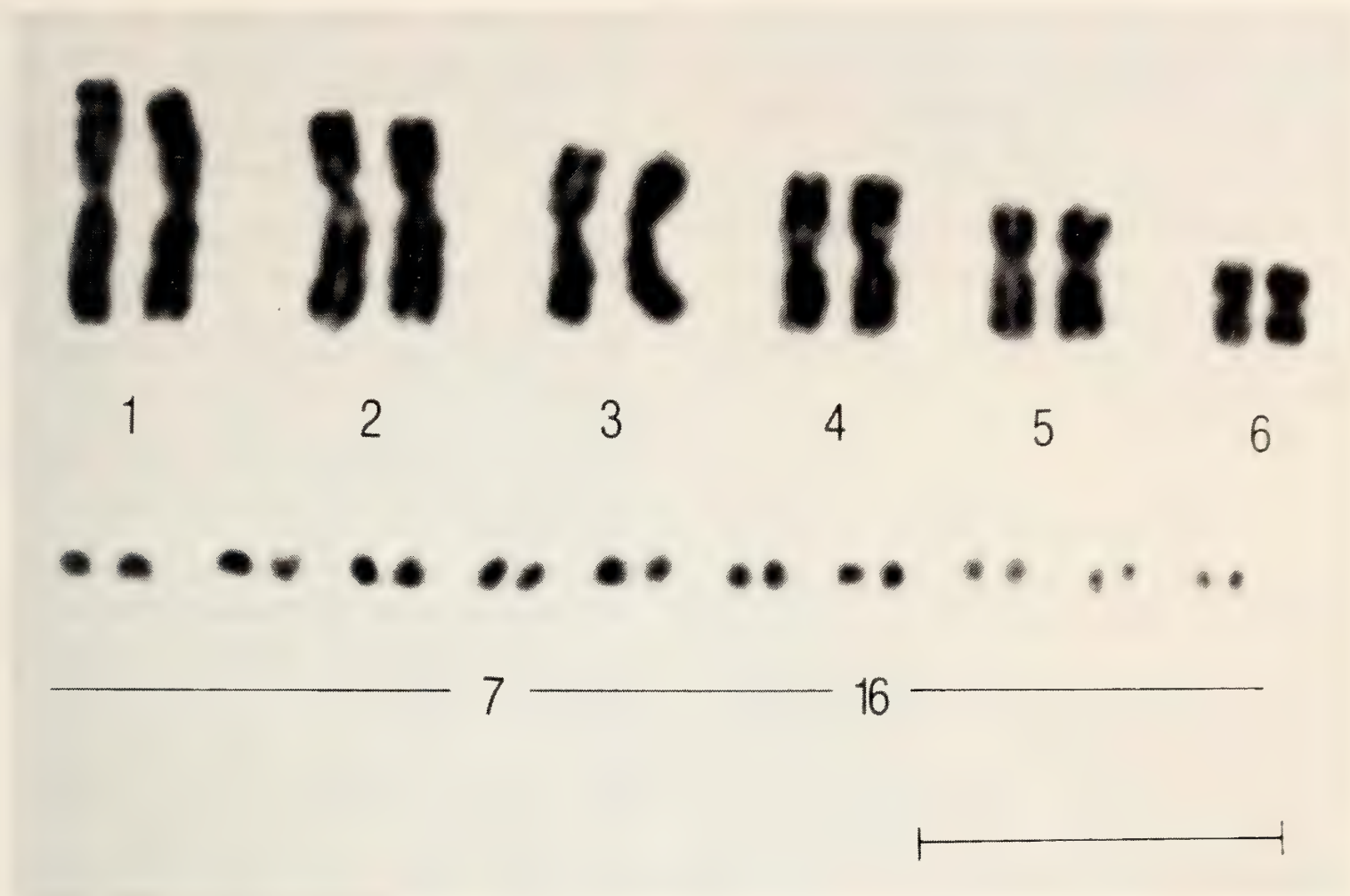


FIG. 3. Karyotype of *Gonocephalus robinsonii*. Bar equals 5 μ m.

their monophyly, presumably along with some other species of the genus not yet studied karyologically.

The karyotype of *G. robinsonii* is similar to karyomorph (1), and considering remarkable differences in the diploid number of chromosomes or the arm number of macrochromosomes between this and other congeneric karyotypes (Table 2), it is unlikely that the karyotype of *G. robinsonii* directly arose from other congeneric karyotypes or *vice versa*. Thus, we conclude that the inclusion of this species in *Gonocephalus* would render the genus paraphyletic.

The number of microchromosomes (20) in the *G. robinsonii* karyotype is smaller than that in the typical agamid karyomorph (1)(22 or 24: Bickham, 1984; King, 1981). Such a chromosomal arrangement (i.e., 12M+20m) is exclusively shared with several agamid species that are supposedly derived from the Australian endemic radiation (Witten, 1983). It is thus probable that

G. robinsonii actually represents dispersals from the Australian Region into Southeast Asia like *Physignathus cocincinus* (see Honda et al., 2000). However, it is also probable that the karyotype of *G. robinsonii* is derived from karyomorph (1), exhibited by a number of other agamid species including several from Southeast Asia (see Ota and Hikida [1989]), through deletions of one or two microchromosome pairs. More comprehensive analyses using biochemical and molecular approaches are needed to determine the relationship of this enigmatic species with certainty.

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Validity of Back-calculation Methods of Body Size from Phalangeal Bones: An Assessment Using Data for *Rana japonica*

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Abstract: Lines of arrested growth (LAGs) appearing in bone sections are useful for age estimation. They also indicate the past growth process in amphibians in temperate zones. Several back-calculation formulae (BCFs) use LAGs to estimate an individual's body size at an earlier time based on the current body size. In order to evaluate the validity of these BCFs, we conducted a mark-recapture and skeletochronological study of female *Rana japonica* in Higashi-Hiroshima, Japan, from 1995 to 1999. The body sizes of 31 recaptured frogs were back-calculated using eight different BCFs and were compared with the frogs' actual body sizes as measured at the previous capture. The most accurate estimation was made by the simplest BCF (Dahl-Lea method) without any regressions between body size and bone diameter; that is, $L_i = L_c(D_i/D_c)$ (L : snout-vent length, D : bone diameter, c : at the time of capture [recapture], i : at the i -th winter).

Key words: Skeletochronology; Body size; Back-calculation formula; *Rana japonica*

INTRODUCTION

In the study of life history and population dynamics, it is important to know the processes of growth and sexual maturation. However, it is difficult to follow an individual organism's growth throughout its life by the mark-recapture method, especially in the case of long-lived species such as some amphibians.

Skeletochronology has proven to be an effective method of age estimation for amphibians in the temperate zone (see Halliday and Verrel, 1988; Castanet and Smirina, 1990). Lines of arrested growth (LAGs) are formed annually in the bone at periods of growth retardation such as hibernation. Thus, LAGs provide valuable information not only on the organism's age (the number of winter seasons a given individual has experienced), but also on the process of bone growth with age (Hemelaar, 1988; Fretey and Le Garff, 1992; Neveu, 1992) and sexual maturation (Francillon-Vieillot et al., 1990; Augert, 1992; Augert and Joly, 1993;

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Caetano and Castanet, 1993; Kusano et al., 1991, 1995a, b).

Since bone growth is considered to reflect growth in body size, body size at an earlier time can be back-calculated from the size of the zone encompassed by a LAG and the current body size (Smirina, 1983; Ryser, 1988; Augert, 1992; Neveu, 1992; Augert and Joly, 1993; Caetano and Leclair, 1996; Leclair and Laurin, 1996). Several different back-calculation formulae (BCFs) have been proposed for the estimation of body size at a past time based on a certain linear relationship between bone size and body size (see Francis, 1990; Ricker, 1992). Although Ryser (1988) commented briefly on the validity of two BCFs for frogs, the validity of these BCFs has not yet been evaluated sufficiently for amphibian species.

We conducted a mark-recapture and skeletochronological study on a population of the Japanese brown frog, *Rana japonica*. Applying different BCFs to recaptured frogs, their body sizes at the previous capture were estimated, and the back-calculated sizes were compared with the actual sizes in order to assess the accuracy of the BCFs. Here, we report the results of the evaluation and discuss the validity of these BCFs as applied to frogs.

MATERIALS AND METHODS

The Japanese brown frog, *Rana japonica*, is commonly distributed in Honshu, Shikoku, and Kyushu, and breeds from January to April in still waters such as rice fields, marshes, and small pools (Maeda and Matsui, 1989). We studied a population of *R. japonica* at "Hiroshima University Ecological Garden" (34°24' N, 132°43' E, altitude 220 m) within the campus of Hiroshima University in Higashi-Hiroshima, Hiroshima Prefecture, Japan.

The mark-recapture study was conducted chiefly during the breeding seasons (January-March) from 1995 to 1999. Since 1996, the survey has also been conducted in a

non-breeding period as well (September-November). Frogs were captured by hand or using a dip net. They were then measured for snout-vent length (SVL) to the nearest 1 mm with a slide caliper, and sexed on the basis of secondary sexual characteristics such as the development of thumbpads and ovaries. The frogs were individually marked by toe-clipping, and were released at the capture sites. Group marking was applied to juveniles newly metamorphosed in 1997-1998, since they were too small to be individually marked. When a frog was captured, the fourth toe of the left or right hind leg was clipped off for a skeletochronological study. Each toe was decalcified in 6% nitric acid for 40 min and washed in running tap water for 24 h. After being embedded in paraffin, the second phalanx of each toe was sectioned (15 μ m thick) and stained with Lili-Meyer's hematoxylin. We selected the best cross-section for each individual, and measured the major and minor axes of the outer margin of the bone and LAGs using an ocular micrometer attached to a light microscope. Originally we used two kinds of bone diameter: the length of the major axis, and the geometric mean of the major and minor axes. Since the results of the analyses using each kind of diameter were quite similar, we report here the results of the analysis using the major axis only.

We assumed that the SVL and phalangeal size of individuals captured in autumn would be nearly equal to those in hibernation, because the mark-recapture study showed that most of the frogs did not show any apparent growth until the next spring. Therefore, we pooled data for yearlings captured in late autumn with those for one-year-olds captured in spring for the analysis. In an earlier study, Francis (1990) reviewed the literature on the calculation of fish lengths at successive ages from growth marks on hard parts of the body, such as scales and otoliths, and he presented several typical BCFs that had been applied in past

researches. Although Francis (1990) recommended an ordinary regression (OR), Ricker (1992) proposed that the geometric mean regression (GMR, also called the reduced major axis) between bone diameter and body size be used as a regression equation in BCFs. In the present study, both types of regressions were used in BCFs. Based on the earlier researches, we applied the following eight BCFs to *R. japonica* (see Francis, 1990; Ricker, 1992).

(1) Dahl-Lea method: $L_i = L_c D_i / D_c$

(2) Regression method :

(2-1) $L_i = p + q D_i$ (OR)

(2-2) $L_i = u + v D_i$ (GMR)

(3) Fraser-Lee method :

(3-1-1) $L_i = (L_c + a/b) D_i / D_c - a/b$ (SPH method using OR; Francis, 1990)

(3-1-2) $L_i = (L_c - p) D_i / D_c + p$ (OR)

(3-2) $L_i = (L_c - u) D_i / D_c + u$ (GMR)

(4) Whitney-Carlander method :

(4-1) $L_i = L_c (p + q D_i) / (p + q D_c)$ (BPH method using OR; Francis, 1990)

(4-2) $L_i = L_c (u + v D_i) / (u + v D_c)$ (GMR)

L: SVL in mm; D: bone diameter in μm ;
c: at capture (recapture in the present study); i: at i-th winter; a, b: D-intercept and slope, respectively, from the ordinary regression equation, $D = a + bL$; p, q: L-intercept and slope, respectively, from the ordinary regression equation, $L = p + qD$; u, v: L-intercept and slope, respectively, from the geometric mean regression (GMR) equation, $L = u + vD$.

We used three regressions between SVL and bone diameter in this analysis, as mentioned above, and these regression equations were calculated from the sample of 74 females in the breeding seasons of 1995 and 1996 and eight juveniles in the autumn of 1996 (see Fig. 1). For recaptured frogs, their body sizes at first capture were estimated using different BCFs, and the back-calculated sizes were compared with the actual sizes. For each BCF, the difference between the back-calculated sizes and the ac-

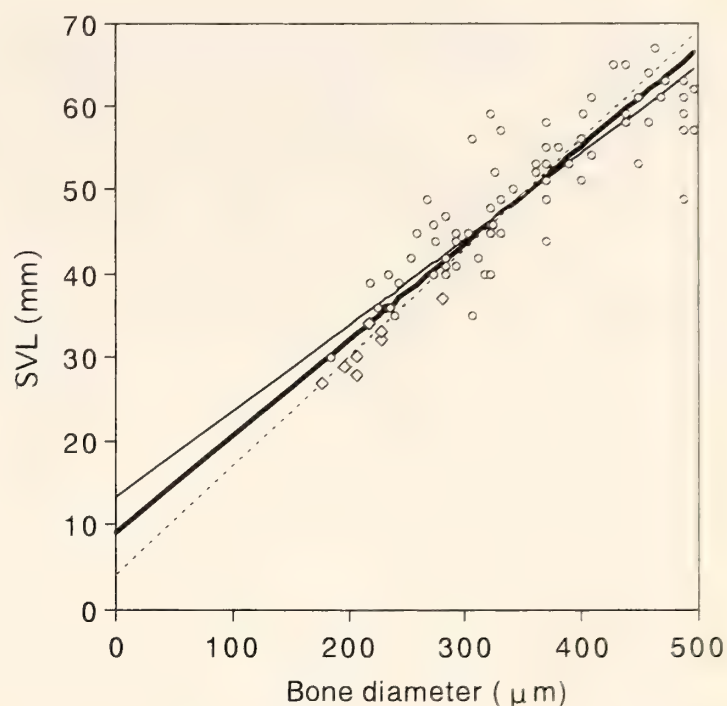


FIG. 1. The relationship between snout-vent lengths (SVL or L) and bone diameter (D) of phalanges. Open circles and squares indicate adults and juveniles, respectively. Three types of regressions are shown: the dotted line is an ordinary regression (OR) of D on L, $D = -32.77 + 7.72L$ ($r = 0.89$, $p < 0.001$); the narrower solid line is an OR of L on D, $L = 13.43 + 0.10D$; the broader line is a geometric mean regression (GMR), $L = 9.11 + 0.12D$.

tual sizes was evaluated using the paired t-test at a significance level of 5%. Since the main purpose of this study was to detect the estimate error in order to evaluate the accuracy of BCFs, we placed a high value on the power of the test by minimizing the type II error rather than the type I error (Sokal and Rohlf, 1995; Jaeger and Halliday, 1998). Therefore, we did not adjust p-values, even when multiple tests were performed.

RESULTS

During the study period, a total of 31 females were recaptured between seasons, but very few males were recaptured. Therefore, we report only on the results for the females. The 31 recaptured females had first been captured in the period between February, 1996, and February, 1999, and

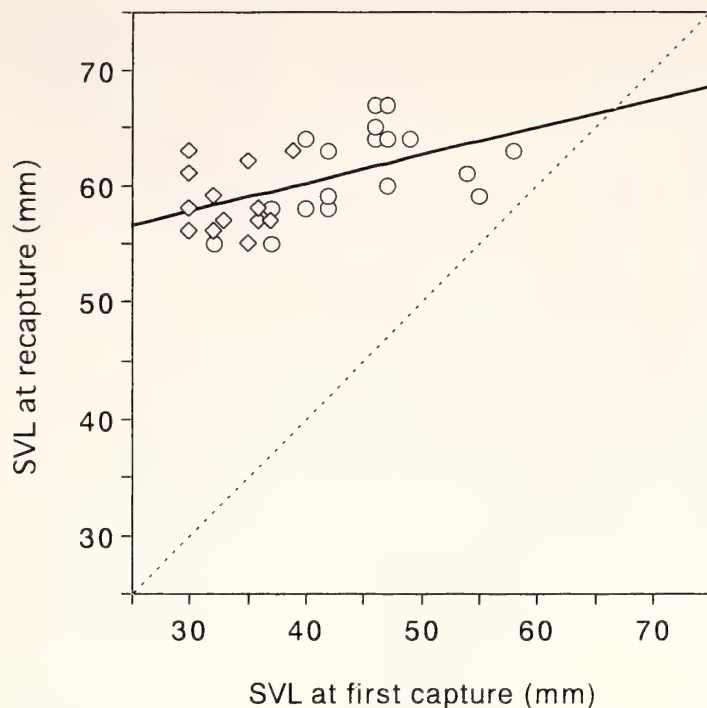


FIG. 2. The relationship between SVLs at recapture (R) and at first capture (F). The solid line indicates the regression line, $R = 50.53 + 0.24F$ ($r = 0.53$, $p = 0.002$) and the dotted line is an isline of $F = R$.

they were recaptured between February, 1997, and September, 1999. No individuals experienced more than one active season between first capture and recapture. Their SVLs ranged from 30 to 58 mm (mean \pm SD, 40.1 ± 7.7) at first capture, and from 55 to 67 mm (60.2 ± 3.5) at recapture (Fig. 2).

Twenty-eight of the 31 frogs were two years old, and the other three frogs were three years old at recapture. At first capture, 18 frogs were sexually mature, whereas 13 were juveniles. In five of the 18 adults and in all 13 juveniles, phalangeal bones were obtained only at recapture, and skeletochronological observation of cross-sections showed that they all were two years old at the time of recapture. In the other 13 adults, phalanges were collected at both first and second captures. These adults had one more LAG at recapture than they had at

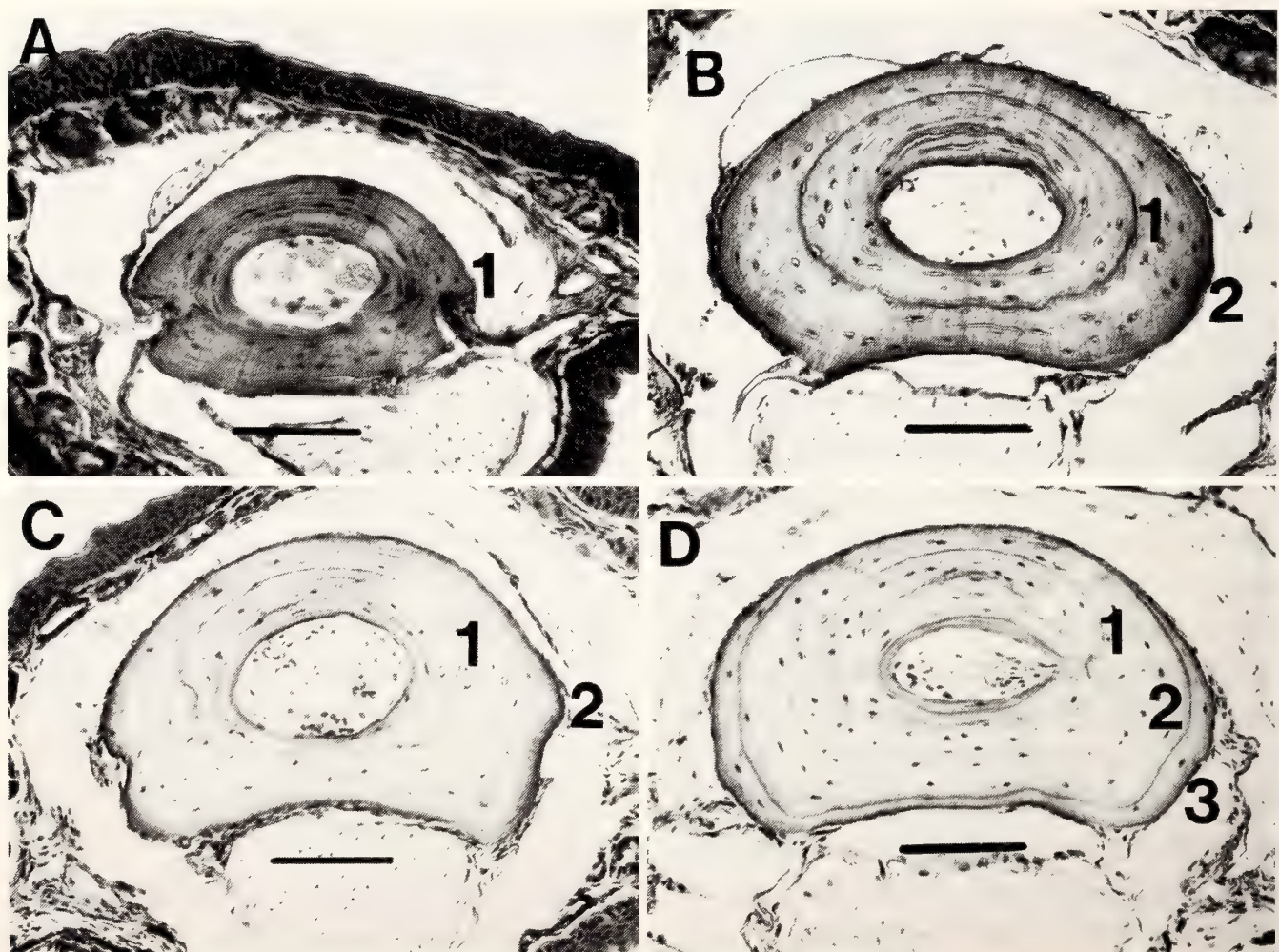


FIG. 3. Cross-sections of phalanges at first capture (left) and recapture (right). The black bar in each photograph indicates a scale of 0.1 mm. (A): A one-year-old female of 40 mm SVL on 11 March 1998. (B): The same individual as (A) measuring 58 mm SVL on 10 February 1999. (C): A two-year-old female of 55 mm SVL on 8 February 1996. (D) The same individual as (C) measuring 59 mm SVL on 16 February 1997.

first capture, which corresponded to a single hibernation (Fig. 3).

The mark-recapture data for these females were analyzed in order to assess the accuracy of the BCFs. Table 1 shows the results of the comparisons between the back-calculated sizes and the actual sizes. The mean SVLs back-calculated by respective BCFs ranged from 40.7 to 45.2 mm, and were more or less larger than the actual value of 40.1 mm (Table 1). The smallest mean of absolute error in the BCFs was 2.3 mm in BCF (1), and the largest value was 5.2 mm in BCF (4-1). Significant differences were detected between back-calculated and actual SVLs in all BCFs except BCF (1) (paired t-test, $p<0.05$). The results showed that among the eight BCFs, BCF (1) gave the most accurate estimate, although it was the simplest equation and did not employ any regressions.

The relationships between the estimates

and the actual SVLs are shown in Fig. 4. Only according to BCF (1), which was the best BCF among those examined (see above), the regression between the estimates (F_e) and actual SVLs (F) was not significantly different from the isoline of $y=x$. The regressions for other BCFs exhibited significant deviations from the isoline. In BCFs (3) and (4), the estimate error became larger when the SVL was smaller, while the estimates were relatively close to actual values at large SVLs. On the other hand, the estimate errors increased at both extreme values of SVL, and were relatively small at medium SVLs in BCF (2) (Fig. 4). In all BCFs except for BCF (1), there were significant negative correlations between estimate error (E) and actual SVL at first capture (F), r_{FE} ($p<0.05$, Table 2). We analyzed other correlations among the variables E , F , actual SVL at recapture (R), and growth (G) to determine the estimate biases

TABLE 1. Results of back-calculation of snout-vent lengths (SVLs) at the previous capture based on eight back-calculation formulae (BCFs). The difference between estimated SVLs and actual SVLs are tested by the paired t-test. Asterisks denote statistically significant differences ($p<0.05$).

BCF	SVLs (mm) $\bar{x}\pm SD$ (range)	Ratio $\bar{x}\pm SD$ (range)	Error (mm) $\bar{x}\pm SD$ (range)	Absolute error (mm) $\bar{x}\pm SD$ (range)	Paired t-test p-value
Actual value	40.1 \pm 7.7 (30-58)				
(1)	40.7 \pm 7.4 2(29-57)	1.02 \pm 0.08 (0.89-0.22)	0.6 \pm 2.8 (-4-7)	2.3 \pm 1.7 (0-7)	0.213
(2-1)	42.7 \pm 5.4 (36-57)	1.08 \pm 0.10 (0.88-1.30)	2.6 \pm 3.6 (-6-9)	3.8 \pm 2.4 (1-9)	<0.001*
(2-2)	42.1 \pm 6.1 (34-58)	1.06 \pm 0.09 (0.86-1.27)	2.0 \pm 3.4 (-7-8)	3.2 \pm 2.4 (0-8)	0.003*
(3-1-1)	42.0 \pm 6.9 (31-57)	1.06 \pm 0.08 (0.91-1.25)	1.9 \pm 2.8 (-3-8)	2.8 \pm 2.0 (0-8)	0.001*
(3-1-2)	45.0 \pm 5.9 (35-58)	1.14 \pm 0.10 (0.98-1.37)	4.9 \pm 3.2 (-1-11)	5.0 \pm 3.1 (0-11)	<0.001*
(3-2)	43.5 \pm 6.4 (33-57)	1.10 \pm 0.09 (0.98-1.31)	3.5 \pm 2.9 (-1-10)	3.7 \pm 2.7 (0-10)	<0.001*
(4-1)	45.2 \pm 6.1 (35-58)	1.14 \pm 0.11 (0.98-1.37)	5.2 \pm 3.3 (-1-11)	5.2 \pm 3.2 (0-11)	<0.001*
(4-2)	43.6 \pm 6.5 (33-58)	1.10 \pm 0.09 (0.97-1.31)	3.6 \pm 3.0 (-1-10)	3.8 \pm 2.7 (0-10)	<0.001*

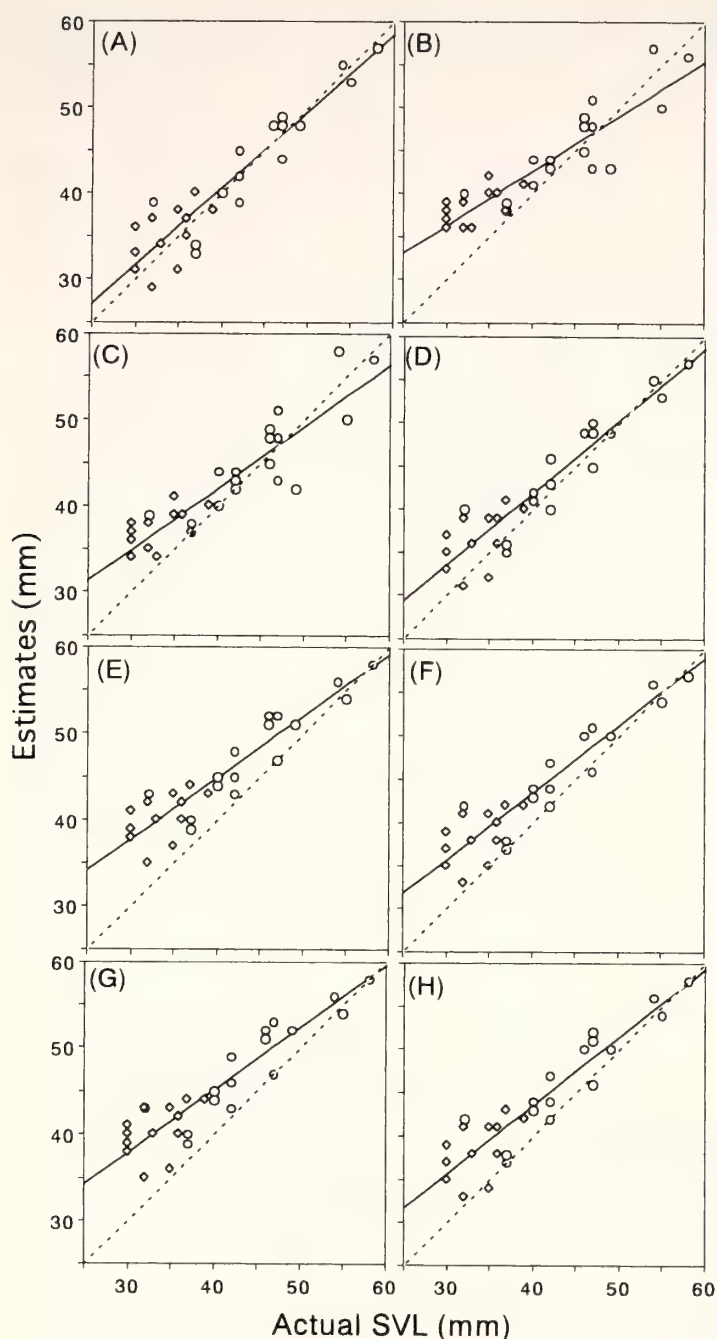


FIG. 4. The relationship between back-calculated (F_e) and actual SVLs (F). Open circles and squares indicate adults and juveniles at first capture, respectively. Solid lines indicate regression lines of F_e against F and dotted lines are isolines of $F_e = F$. (A): BCF(1) $F_e = 4.98 + 0.89F$ ($r = 0.93$, $p < 0.001$); (B): BCF(2-1) $F_e = 17.17 + 0.64F$ ($r = 0.90$, $p < 0.001$); (C): BCF(2-2) $F_e = 13.55 + 0.71F$ ($r = 0.90$, $p < 0.001$); (D): BCF(3-2-1) $F_e = 8.66 + 0.83F$ ($r = 0.93$, $p < 0.001$); (E): BCF(3-2-2), $F_e = 16.50 + 0.71F$ ($r = 0.93$, $p < 0.001$); (F): BCF(3-2), $F_e = 12.57 + 0.77F$ ($r = 0.93$, $p < 0.001$); (G): BCF(4-1), $F_e = 16.36 + 0.72F$ ($r = 0.91$, $p < 0.001$); (H): BCF(4-2) $F_e = 12.17 + 0.79F$ ($r = 0.93$, $p < 0.001$). See text for abbreviations for back-calculation formulae.

of the respective BCFs (Table 2). In BCF (2-1), r_{RE} was significantly negative, indicating Lee's phenomenon in which the larger

individuals at the last capture were estimated to be smaller at any past age (Ricker, 1992). In all of the BCFs, a positive r_{GE} ($p < 0.05$) showed that the estimates of past SVLs of fast-growing individuals were larger than actual. However there was a significantly positive correlation between R and F ($r_{FR} = 0.53$, $p = 0.002$, Fig. 2), and a negative correlation between G and F ($r_{FG} = -0.89$, $p < 0.001$). Therefore, we also analyzed partial correlations among these variables.

In all BCFs, the partial correlations controlled for R ($r_{FE.R}$) were significantly negative ($p < 0.05$). In contrast to r_{RE} , the partial correlation $r_{RE.F}$ indicated the inverse of Lee's phenomenon in BCFs (1), (3), and (4). These BCFs also showed the tendency to overestimate past SVLs of fast-growing individuals (significantly positive $r_{GE.F}$). The Dahl-Lea, Fraser-Lee, and Whitney-Carlender methods were thus affected by the size of the frog at last capture and by growth.

We collected as many juveniles as possible and measured their SVLs in autumn of 1996 and 1997, in order to determine the minimum SVL at sexual maturity. Their SVLs ranged from 24 to 40 mm, with the mean being 31.0 (SD = 3.3, $N = 99$). Since the upper limit of the 95% range based on t-distribution was 37.4 mm when back-calculated SVLs were 37 mm or less, the frogs were estimated to have been juveniles at the time of capture. The number of individuals estimated to have been juveniles based on back-calculated SVLs varied greatly from 2-10 depending on the BCF used (Table 3). BCF (1) gave a value of 10, and its rate of correct judgement was 0.83 as a whole, the best performance among the BCFs examined. The other BCFs gave very poor judgement of juveniles that were less than half of the actual number in most cases. BCFs (3-1-2) and (4-1) gave the lowest estimate of only two. The BCFs other than BCF (1) are considered to be ineffective in providing this estimate.

TABLE 2. Correlations between estimate errors and SVLs. Asterisks show significant correlations ($p<0.05$). Symbols of variables are: F, actual SVL at first capture; R, actual SVL at recapture; G, growth, i.e., difference of SVL between captures; E: estimate error, i.e., difference between estimates and actual SVL at first capture.

BCF	r_{FE}	$r_{FE.R}$	r_{RE}	$r_{RE.F}$	r_{GE}	$r_{GE.F}$
(1)	-0.30	-0.45*	0.14	0.37*	0.43*	0.37*
(2-1)	-0.77*	-0.72*	-0.38*	0.04	0.70*	0.04
(2-2)	-0.65*	-0.60*	-0.30	0.06	0.60*	0.06
(3-1-1)	-0.46*	-0.60*	0.09	0.44*	0.59*	0.44*
(3-1-2)	-0.71*	-0.80*	-0.06	0.52*	0.80*	0.52*
(3-2)	-0.59*	-0.70*	0.00	0.46*	0.70*	0.46*
(4-1)	-0.66*	-0.79*	0.02	0.57*	0.78*	0.57*
(4-2)	-0.55*	-0.67*	0.02	0.44*	0.66*	0.44*

TABLE 3. Validity of determination of sexual maturity based on back-calculated SVLs.

BCF	N of individuals judged correctly as juveniles	N of individuals judged correctly as adults	Rate of correct judgements
Actual N	13	18	—
(1)	10	16	0.83 (26/31)
(2-1)	4	18	0.71 (22/31)
(2-2)	6	17	0.74 (23/31)
(3-1-1)	8	16	0.77 (24/31)
(3-1-2)	2	18	0.65 (20/31)
(3-2)	5	17	0.71 (22/31)
(4-1)	2	18	0.65 (20/31)
(4-2)	5	17	0.71 (22/31)

DISCUSSION

In almost all of the phalangeal cross-sections of *R. japonica*, stained LAGs were distinct, being easily counted and measured (Fig. 3). The mark-recapture procedure showed that skeletochronology was an effective age-determination method in this species in addition to some other amphibian species that had been studied previously, such as *Bufo bufo* (Hemalaar and Van Gelder, 1980; Fretey and Le Garff, 1992), *R. temporaria* (Gibbons and McCarthy, 1983), and *B. calamita* (Tejedo et al., 1997). Francis (1990), based on the survey of a number of relevant papers on fish popula-

tions, demonstrated that the Fraser-Lee method (BCF (3)) was the most popular, followed by the regression (BCF (2)) and Dahl-Lea (BCF (1)) methods in that order. Ricker (1992) evaluated the validity of BCFs on theoretical grounds, and argued that the regression between scale (bone) diameter and body length for BCFs must be GMR rather than OR, and that the Fraser-Lee method (BCF (3-2)) is preferable. Similar studies of amphibian species include those of Kusano et al. (1991), who applied the Dahl-Lea method (BCF (1)) to *B. marinus*, and Augert and Jolly (1993), who used the regression method (BCF (2-1)) for *R. temporaria*. The Fraser-Lee method

has also been used for back-calculation in amphibian species. For example, BCF (3-1-2) has been applied to *B. bufo* (Smirina, 1983), *R. septentrionaris* (Leclair and Laurin, 1996) and *Notophthalmus viridescens* (Caetano and Leclair, 1996). Neveu (1992) reported that the mean body size of a certain age group, which was estimated by the Whitney-Carlander method (BCF (4-1)), did not differ from that derived from the regression method (BCF (2-1)) for *R. esculenta*. In *R. temporaria*, Ryser (1988) used not only an ordinary regression line between SVL and phalangeal diameter, but also a specially devised linear equation. He assessed the accuracy of BCF (2-1) and BCF (4-1) by comparing values estimated by these methods and actual values from mark-recaptures. His results suggested that BCF (4-1) was preferable, since the mean of absolute error was 2.13 mm as compared to 3.13 mm for BCF (2-1).

In the present study, the accuracy was compared between the BCFs incorporating OR and GMR as regression procedures: e.g., between (2-1) and (2-2), (3-1-2) and (3-2), and (4-1) and (4-2). The results showed that BCFs employing GMR give more accurate estimates than those using OR (Table 1). This supports Ricker's (1992) claim that GMR is preferable to OR for BCFs. The Fraser-Lee method (BCF (3)), exclusive of the SPH option (BCF (3-1-1)), and the Whitney-Carlander method (BCF (4)) made larger errors than BCF (2), regardless of regression methods. BCF(1) is just a special form of BCFs (3) and (4): it is identical to BCFs (3) and (4) if y-intercepts (a, p, and u) are fixed at zero. Standard regression theory shows that $-a/b$ is always less than p (Francis, 1990). Therefore, the estimates made by BCF (3-1-1) were nearer to those of BCF (1) than of BCF (3-1-2), but BCF (3-1-1) was still less accurate than BCF (1). Therefore, at least for *R. japonica*, the relationship between SVL and phalangeal diameter seems to be inappropriate for the "scale proportional

hypophysis (SPH)" and the "body proportional hypothesis (BPH)" as had been suggested by Francis (1990) for fishes.

In the present study, the best BCF among the eight different BCFs examined proved to be the simplest formula using the Dahl-Lee method (BCF (1)) (Table 1). All of the other BCFs used the regression equations between bone diameter and SVL: only BCF (1) did not require such regressions. Despite such simplicity, BCF (1) gave the most accurate estimates. The mean of absolute error for BCF (1) was 2.3 mm, only about 6% of the actual SVL (Table 1). The formula's simplicity is also advantageous for its application to population studies in the field. Although a large sample of target animals with a wide range of body sizes or ages is needed to calculate reliable regressions, BCF (1) does not require such a large sample. This BCF can be applied even to a small sample and still produce estimates with high accuracy.

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Relationship Between Brightness or Size and the Presence of Barnacles on the Carapace of the Hawksbill Turtles (*Eretmochelys imbricata*)

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Abstract: In this study, by converting the colors of the first coastal scutes (C1) in the hawksbill turtle, *Eretmochelys imbricata*, into numerical values by the shade (256 phases), it became possible to fully analyze the brightness of the carapace. Two thousand seven hundred fifty-six C1s of hawksbill turtles that had been captured in the Cuban sea from 1993 through 1994 were used. The relationship between the brightness and the width of the first costal (C1W) was examined. The results showed no correlation between brightness and C1W. However, C1 with barnacles tends to have greater C1W (27.1 ± 2.8 cm), while no barnacles were found on C1 with a width of 20.3 cm or less. C1 with barnacles, compared to C1 without, was low in brightness (somewhat dark) in terms of statistical significance.

Key words: Hawksbill turtle; Barnacle; Brightness; Scute; Size

INTRODUCTION

It has been reported that changes in pattern as the carapace grows help to roughly classify the sex or life stages of several turtle species (Barbour and Carr, 1940; Moll, 1980). Although it is not clear whether this fact is linked to the age of the turtle, it is highly likely that it is related to the sex and size (McCoy, 1968; Balazs, 1986). There have been no studies on this relationship in sea turtles, because it is difficult to track turtles in the sea to study their carapace patterns. While the carapace is made of hard tissues and very likely to remain after death from a cause like stranding. So it is interesting to obtain new information on its color or pattern from the carapace.

In this study, the C1 brightness of hawksbill turtles, *Eretmochelys imbricata*, living in the Cuban sea was converted into numerical values by 256 phases of shade, and then its relationship to the width of the first costal (C1W) was examined. Next, between the turtle groups with barnacles or not, the relationship between C1W and the brightness was studied. There has been no information on either the size or the brightness of the hawksbill carapace. Not only that, there have been no reports on the relation of barnacles attached to the carapace and the brightness of the carapace.

MATERIALS AND METHODS

I used the right first costal (C1) of

hawksbills ($N=2,756$) that were captured using fishing top nets of 46–53 cm mesh, 50–60 fathoms long, 12–15 meshes deep, from 1993 to 1994. The sex, carapace length, and other information from each turtles were unknown. The reason for selecting C1 is that C1 is very distinct from all other scutes in shape, and therefore it is easy to distinguish. Furthermore, by using only the left side of C1, a redundant use of turtles can be avoided.

I first studied the relationship between the brightness of C1 and C1W was examined. Then, for individual groups, whether with or without barnacles (*Chelonibia*), I studied the relationships between C1W and the brightness. Regarding barnacles on loggerheads (*Caretta caretta*), there are reports that they attached themselves to the dorsal carapace more than the ventral carapace (Gramentz, 1988) and attachment on the ventral carapace could be seen on the anterior, posterior, and middle vertebral scutes (Matsuura and Nakamura, 1993). But since hawksbills have the habit of digging under a coral reef and it is considered that barnacles on the middle vertebral scutes can be easily scraped off, I chose C1 to examine whether there were attached barnacles or not. The two groups were defined as turtles in which traces of attached barnacles could be observed, and turtles with barnacles attached to them.

Brightness of C1

Colors are generally classified by the three basic characteristics of hue, intensity, and brightness. Hue is expressed by the wavelength of light. Intensity indicates the degree of saturation or dullness of the color and is expressed by the amount of gray proportionate to hue. Brightness indicates the degree of shade of the color ranging from 0% (black) to 100% (white). In this study, I divided the degree of brightness into 256 computer-classifiable phases, converting C1 into numerical values of shade.

With the newly formed scute side facing

upwards, photographs of C1 were shot in a display case and a spot light on two separate days under same shooting and film-developing conditions. Using a scanner, the photographs were converted into image data and the brightness of C1 was converted into numerical values using the degree of shade (256 phases). In other words, the numerical value of shade was high on the amber-colored part while the black speckles were low in the numerical value of shade. I used Image8.3 by Erdas (Inc.) for analyzing the shade of the colors. For all the turtles, I determined the mean brightness ("brightness" hereafter) of the entire C1 area.

C1W

C1W was individually measured using a tape measure (± 0.1 cm).

Statistical treatment

The correlation between the C1 brightness and the C1W was examined using regression lines. The relationships between the classification depending on the presence of barnacles, C1W, and the brightness were clarified by a one-way layout ANOVA without assuming homogeneity.

RESULTS

Relationship between brightness and C1W

No correlation was observed between the brightness and C1W (Fig. 1). The regression slope was $Y=0.3845X+92.133$ and r^2 was 0.0026.

Relationships between the presence of attached barnacles and C1W or the brightness

It became clear that there is no correlation between the brightness and C1W (Fig. 1). Thus, I compared C1W and the brightness between the groups with ($N=669$) and without barnacles ($N=2087$), through a one-way layout ANOVA (Tamhane) without assuming homogeneity. The results showed that there was a significant difference in C1W depending on the

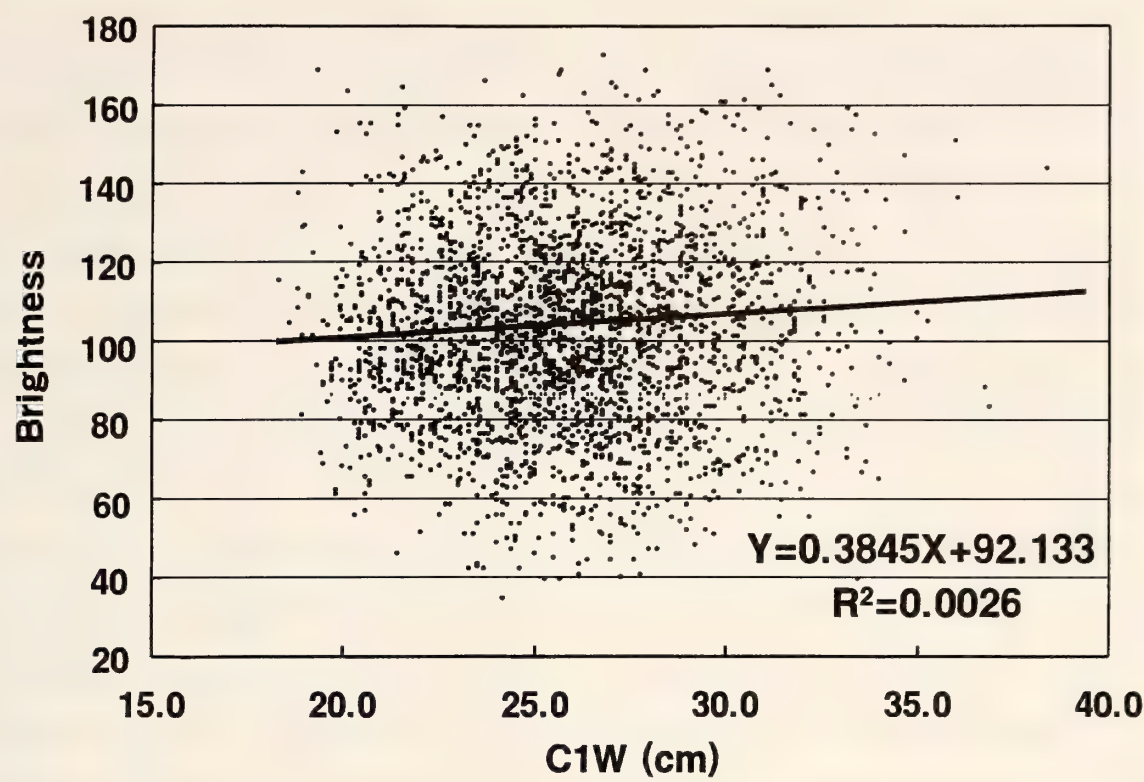


FIG. 1. Scatter plots of C1W (in cm) and brightness (in 256 phases, N=2,756).

presence of barnacles ($F=135.1$, $p<0.01$). The mean C1W with barnacles was 27.1 ± 2.8 cm while without barnacles the mean was 25.5 ± 3.2 cm. The frequency distribution of C1W with or without barnacles is clearly different (Fig. 2). C1W of 20.3 cm or smaller showed no barnacles. In the same manner, the relationship between

barnacles and the brightness indicated that, compared to others, C1 with barnacles is significantly low in brightness (somewhat dark) ($F=22.2$, $p<0.01$). The mean brightness of C1 with barnacles was 98.3 ± 25.5 and the rest was 103.3 ± 23.6 .

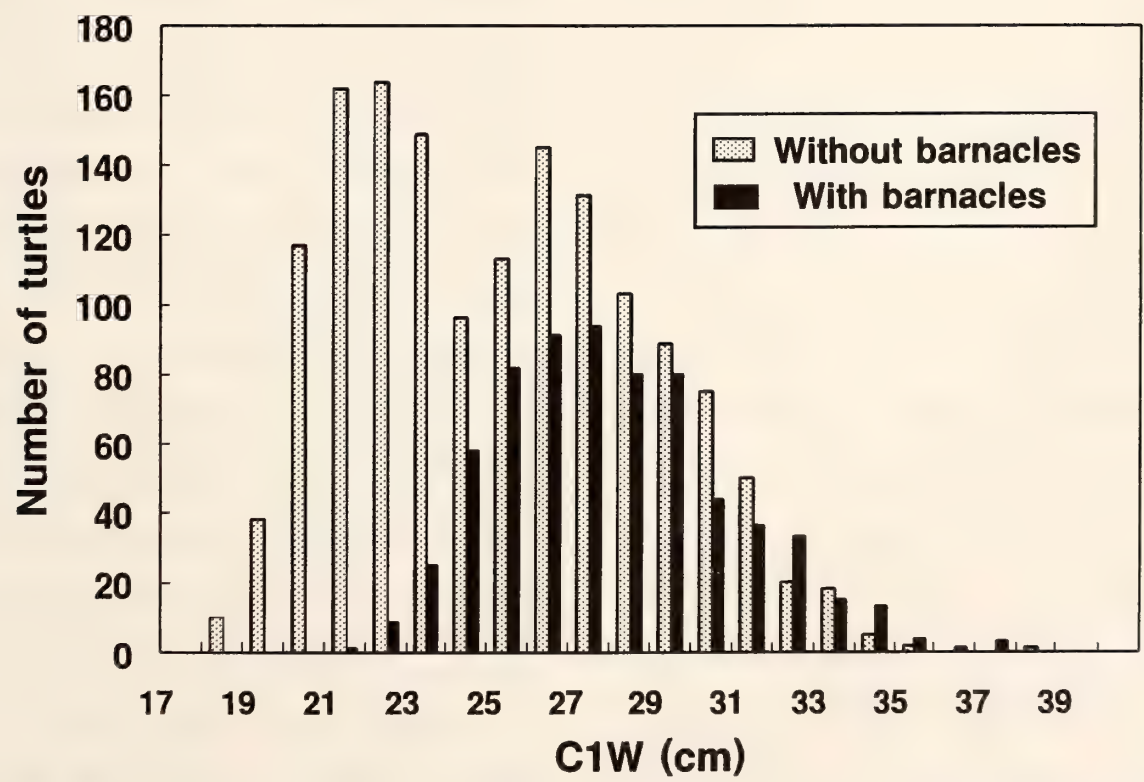


FIG. 2. C1W distribution of turtles with (dark rectangles) and without (gray rectangles) barnacles.

DISCUSSION

Melanin that has been accumulated on melanocytes is converted into granule cell melanosomes, and then shifts to the neighboring cell keratinocytes that produce hawksbill scutes, gradually forming a carapace through keratinization. When this happens, the black and amber colors shift to keratinocytes simultaneously. Since intracellular melanin consists of eumelanin of black pigment (Seiji, 1970; Oikawa, 1976) and pheomelanin of yellow-to-red pigment (Prota, 1980), the former is considered to make black speckles, and the latter amber areas, thereby giving different colors to the carapace. The common element between the two is melanin-producing melanocytes, but it is not known exactly which is induced by what stimulation. A common theory holds that they are controlled by the genes (an intrinsic element) (Seiji, 1970; Oikawa, 1976; Prota, 1980). The effects of a ray of light (Seiji et al., 1973) or hormones (melanocyte-stimulating, adrenocortical, sex, and thyrotropic hormones) (Seiji, 1970; Parker, 1974; Snell, 1966) can provide the necessary conditions for melanocytes to stimulate the generation of melanosomes (brightness of carapace). Empirically, carapace patterns of the hawksbill are bilaterally symmetrical. Since the way the pattern starts out at individual scutes is identical, it is presumed that those patterns (two types of the melanin arrangement) have more genetic (innate) factors than environmental (acquired) factors. However, when a hawksbill turtle is raised without sunlight, its carapace colors become lighter. Thus, environmental factors are believed to be involved when it comes to brightness.

In general the developmental habitat does not seem to differ from that of the adult, and juveniles and adults are taken together in the same forging areas (Limpus, 1992; Broderick et al., 1994). So those turtles, within the same feeding habitat, were considered sub-adults or adults. In this study,

no correlation was observed between C1W and brightness (Fig. 1). This fact at least indicates that in the case of a hawksbill with a C1W of 18.3 cm ($SCL = 51.3$ cm, calculated by $SCL = 4.3527 (C1W)^{0.8484}$, $r^2 = 0.9529$, $N = 340$, unpublished data) or greater there is little change in scute color as it grows. However, there have been reports of changes in carapace patterns of other turtle species due to sexual differences after maturity (Barbour and Carr, 1940; Moll, 1980), as well as due to life stages and size (McCoy, 1968; Balazs, 1986). Considering those reports, there is a possibility that endogenous substances would change the patterns. Therefore, it may be worth studying the changes in patterns of hawksbills before and after sexual maturity or in relation to the differences of the sexes.

Barnacles that I examined in this study mostly belonged to the genus *Chelonibia* by morphology, although I could not classify them as to species. There is a report that these same barnacles on the carapace are typical of hawksbills on the Caribbean coast of Costa Rica, but not of *Chelonia* (*Chelonia mydas*) (Carr et al., 1966). *Chelonibia*, the commensal barnacle on sea turtles, possesses marine qualities and is gregarious in shallow water (on the coast). Elements that regulate the distribution of Cirripedia including barnacles are temperature and the concentration of salt. Since the embryos scatter seeds while swimming freely, the durability against external conditions does regulate the geographical distribution (Shiino, 1964). With these facts about barnacles in mind and the fact that the greater the C1W the more significant the C1 with barnacles is and that barnacles do not attach to C1 with C1W of 20.3 cm ($SCL = 56.0$ cm) or smaller (Fig. 2), it is highly likely that turtles with barnacles have different migration routes from turtles without barnacles and these turtles are a set of turtles which frequently use shallow water (coast). The fact that C1s with attached barnacles were significantly lower in

brightness (somewhat dark) than other C1s is considered to be caused by the accumulated melanin of exogenous elements (such as the amount of sunlight) after the hawksbills moved to shallow water. In fact, all the species of sea turtles move at least a small distance from the feeding habitat to the breeding habitat. After mating, males return to the feeding habitat while females move to the nesting habitat (Limpus and Miller, 1993). After nesting for several months, females return to the feeding environment, preparing for the next breeding (Limpus and Miller, 1993; Miller, 1985). Also, the feeding habitat is generally the same whether they are baby turtles, sub-adults, or adults (Limpus, 1992; Broderick et al., 1994). In Cuban populations, sexual maturity is reached when SCL is 51–55 cm, the size of the nesting turtle ranges from 60 to 85 cm, and male sexual maturity is reached when SCL is 68 cm or greater (Moncada et al., 1998). What those facts suggest, and what has become clarified in this paper is that a turtle with attached barnacles will move to shallow water or the coast for certain reasons (e.g., mating or nesting) and sexual maturity and the difference between the sexes may be linked to the attachment of barnacles. The hypothesis fits the report that small (SCL < 50 cm) loggerheads do not have these barnacles in the Mediterranean sea (Gramentz, 1988).

I used 256 phases of shade to measure the brightness of hawksbill carapaces. Empirically speaking, because the brightness of the carapace is believed to include environmental elements, it is possible to gather ample information by linking the brightness to other environmental factors. In this study, as an example, with the characteristics of one turtle with attached barnacles I was able to express the brightness and size. Especially, for marine turtles which are difficult to follow and examine in the ocean with the naked eye, it is possible to obtain data of brightness of carapace on stranded turtles or live turtles. Thus, this suggests that in-

formation based on the brightness can add to a fuller understanding of the environmental characteristics of hawksbill turtles.

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Literature Survey on Predators of Snakes in Japan

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Abstract: Characteristic defensive behaviors of snakes and their ecological and morphological correlates have been well documented. Biological interpretations of these characteristics, however, often suffer from a paucity of information on actual predators of snakes. Concerning natural predators of Japanese snakes, neither quantitative data nor a systematic review are available. Here, we review and synthesize the published accounts of predators of snakes in Japan. We confirmed 59 species/subspecies of predators and 21 species of prey snakes. We hope that this review will stimulate biologists and naturalists to record further predatory events on snakes and help clarify the defensive mechanisms of snakes.

Key words: Snake; Predator; Prey; Review; Japan

INTRODUCTION

Prey-predator interaction is one of the most important aspects of evolutionary biology, because this interaction can affect the evolution of phenotypic features, such as morphology and behavior, of both prey and predators (Feder and Lauder, 1986).

Snakes, typical carnivorous animals, have been well-studied as “predators”. Substantial information on their diets, feeding behavior, and associated ecological and morphological characteristics have been accumulated (Mushinsky, 1987; Greene, 1997). Attention has also been paid to snakes as “prey”, and characteristic defensive behaviors and ecological and morphological correlates have been documented (Greene, 1988; Pough, 1988). However,

biological interpretations of their defensive mechanisms often suffer from a paucity of information on actual predators of snakes.

In Japan, 38 species of snakes (excluding sea snakes) are currently recognized (Hikida and Sengoku, 2000). For several species of them, food habits are well documented, and Mori and Moriguchi (1988) reviewed the published data on natural diets of Japanese snakes. On the other hand, information on predators of Japanese snakes are scattered, and neither quantitative data nor a systematic review are available concerning natural predators of snakes in Japan. We have attempted to review and synthesize the published accounts of predators of snakes in Japan, and we provide a list of natural predators of Japanese snakes based on more than 200 references.

We employed the following criteria for citation. 1) The data used here were limited to predatory events observed within Japan. For the snakes that are not endemic to Japan, predatory events observed in other

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countries were not included. 2) Although humans could play an important role in the evolution of phenotypic features of snakes, predation by *Homo sapiens* was not considered. 3) Predation under captive or experimental conditions was excluded. 4) Actual ingestion of the snake by the predator was not considered. For example, observation of a flying bird carrying a snake or observation of a predatory attempt without actual ingestion was considered as a predatory event and included in the present cita-

tion. 5) Predation obviously considered as carcass feeding was excluded. However, unless evidence of carcass feeding was available, predation on dead snakes was included. 6) General descriptions of food habits and speculated predators without concrete evidences were excluded. 7) If the data obviously obtained from the same survey were published in separate papers, we cited only a representative one. 8) Predators of sea snakes were excluded, and we did not make an extensive literature survey for sea snakes.

TABLE 1. List of animals reported as predators of Japanese snakes. Subspecies names of predators are listed if identification is possible from the literature.

Class	Order	Species*	Symbol
Arachnida	Araneae	<i>Achaearanea tepidariorum</i>	Ar-1
		<i>Nephila maculata</i>	Ar-2
Osteichthyes	Anguilliformes	<i>Anguilla marmorata</i>	O-1
	Salmoniformes	<i>Oncorhynchus masou masou</i>	O-2
		<i>Oncorhynchus mykiss</i>	O-3
		<i>Salvelinus leucomaenis pluvius</i>	O-4
		<i>Salvelinus leucomaenis</i>	O-5
Amphibia	Urodela	<i>Andrias japonicus</i>	A-1
	Anura	<i>Bufo</i> sp.	A-2
		<i>Rana catesbeiana</i>	A-3
		<i>Rana nigromaculata</i>	A-4
		<i>Rana ornativentris</i>	A-5
Reptilia	Squamata	<i>Agkistrodon blomhoffii</i>	R-1
		<i>Dinodon orientale</i>	R-2
		<i>Dinodon rufozonatum walli</i>	R-3
		<i>Dinodon semicarinatum</i>	R-4
		<i>Elaphe climacophora</i>	R-5
		<i>Elaphe quadrivirgata</i>	R-6
		<i>Hemibungarus japonicus boettgeri</i>	R-7
		<i>Hemibungarus japonicus japonicus</i>	R-8
		<i>Hemibungarus macclellandi iwasakii</i>	R-9
		<i>Ovophis okinavensis</i>	R-10
		<i>Trimeresurus elegans</i>	R-11
		<i>Trimeresurus flavoviridis</i>	R-12
Aves	Ciconiiformes	<i>Ardea purpurea manilensis</i>	V-1
		<i>Nycticorax nycticorax nycticorax</i>	V-2

TABLE 1. Cotinued.

	Falconiformes	<i>Accipiter gentilis fujiyamae</i>	V-3
		<i>Aquila chrysaetos japonica</i>	V-4
		<i>Butastur indicus</i>	V-5
		<i>Buteo buteo japonicus</i>	V-6
		<i>Circus spilonotus spilonotus</i>	V-7
		<i>Falco tinnunculus interstinctus</i>	V-8
		<i>Haliaeetus albicilla albicilla</i>	V-9
		<i>Milvus migrans lineatus</i>	V-10
		<i>Pernis apivorus orientalis</i>	V-11
		<i>Spilornis cheela perplexus</i>	V-12
		<i>Spizaetus nipalensis orientalis</i>	V-13
	Galliformes	<i>Phasianus colchicus</i>	V-14
		<i>Phasianus soemmerringii</i>	V-15
	Strigiformes	<i>Strix uralensis</i>	V-16
	Coraciiformes	<i>Halcyon coromanda major</i>	V-17
	Passeriformes	<i>Corvus corone orientalis</i>	V-18
		<i>Corvus macrorhynchos japonensis</i>	V-19
		<i>Corvus</i> sp.	V-20
		<i>Lanius bucephalus bucephalus</i>	V-21
		<i>Sturnus cineraceus</i>	V-22
Mammalia	Primates	<i>Macaca fuscata yakui</i>	M-1
	Carnivora	<i>Felis bengalensis euphilura</i>	M-2
		<i>Felis catus</i>	M-3
		<i>Felis iriomotensis</i>	M-4
		<i>Herpestes javanicus</i>	M-5
		<i>Meles meles anakuma</i>	M-6
		<i>Mustela itatsi</i>	M-7
		<i>Mustela sibirica coreana</i>	M-8
		<i>Mustela vison</i>	M-9
		<i>Nyctereutes procyonoides viverrinus</i>	M-10
		<i>Ursus arctos</i>	M-11
		<i>Vulpes vulpes japonica</i>	M-12
		<i>Vulpes vulpes schrencki</i>	M-13
		Unidentified species**	M-14
	Artiodactyla	<i>Sus scrofa riukiuanus</i>	M-15

* Scientific names follow literature listed below. Thus, they are not necessarily consistent with those used in the original literature.
Arachnida, Osteichthyes, and Mammalia: Hidaka, T. (ed.) 1998. The Encyclopaedia of Animals in Japan, Extra Volume, General Index and Red Lists of Threatened Animals in Japan. Heibonsha, Tokyo. 334 p.
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** Authors speculated that the predator was *Canis familiaris* or *Felis catus* based on the feces.

However, a few items of information obtained during our general survey are presented as an appendix.

We attempted to give translations for literature without a original English title and/or English name of the periodical. For the convenience of Japanese readers, a list of the literature in Japanese and the ta-

TABLE 2. List of literature records for predators of Japanese snakes. See Table 1 and Literature List for symbols of predators and reference No., respectively.

Snake species	Predator (reference No.)
<i>Achalinus spinalis</i>	R-1 (23, 76, 103, 104, 131, 197), R-2 (188, 198), R-6 (44, 94, 109, 186) M-3 (187)
<i>Achalinus wernerii</i>	R-4 (171), R-12 (74, 75, 97)
<i>Agkistrodon blomhoffii</i>	O-3 (120) A-2 (176) R-6 (6, 92, 165, 175, 185, 191) V-4 (39, 48, 154*), V-5 (3, 56, 82), V-20 (161) M-7 (16, 119)
<i>Amphiesma pryeri pryeri</i>	R-4 (96), R-10 (73, 95), R-12 (70, 71, 73, 75, 97) M-7 (224), M-14 (210)
<i>Amphiesma vibakari vibakari</i>	R-1 (54, 98, 99), R-2 (147), R-6 (43, 54, 94) V-5 (55**), V-21 (85)
<i>Cyclophiops herminae</i>	V-12 (181) M-4 (136, 137, 138, 205, 218)
<i>Cyclophiops semicarinatus</i>	R-4 (12, 96, 113, 169), R-10 (95), R-12 (69, 70, 71, 73, 75, 97, 171) V-1 (193), V-5 (110) M-5 (2)
<i>Dinodon orientale</i>	R-6 (127) M-3 (32)
<i>Dinodon rufozonatum rufozonatum</i>	M-2 (47, 146)
<i>Dinodon rufozonatum walli</i>	M-4 (136, 137, 138, 139, 205, 218)
<i>Dinodon semicarinatum</i>	Ar-2 (7) R-4 (96), R-10 (95, 182), R-12 (73, 75, 97) M-5 (195)
<i>Elaphe climacophora</i>	Ar-1 (8) A-1 (143), A-4 (151), A-5 (67) R-5 (178, 192), R-6 (25, 44, 197) V-3 (64), V-4 (9, 39, 48, 144, 154*, 155, 157, 160, 163, 164, 174, 180, 183, 204, 209, 212, 214), V-5 (3, 55**, 82, 123), V-6 (59, 211, 221), V-9 (31, 108), V-10 (57), V-13 (19, 48, 111, 112, 200, 206), V-14 (226), V-16 (14), V-19 (84), V-21 (18, 107) M-2 (47, 213), M-3 (116, 213), M-7 (179), M-11 (66)
<i>Elaphe conspicillata</i>	R-6 (190) V-4 (39, 154*), V-5 (3, 123), V-6 (56, 162), V-11 (1), V-13 (200), V-17 (42), V-21 (18)
<i>Elaphe quadrivirgata</i>	A-3 (33)

TABLE 2. Cotinued.

	V-3 (64), V-4 (39, 144, 154*, 163, 164, 174, 209, 214), V-5 (3, 86**, 87, 105, 159), V-6 (36, 52, 221), V-10 (63, 117, 159, 172), V-13 (48, 61, 200), V-21 (58, 106, 114) M-1 (91)
<i>Elaphe taeniura schmackeri</i>	M-4 (136, 137, 138, 139, 205)
<i>Elaphe</i> sp.	V-13 (111)
<i>Hemibungarus japonicus japonicus</i>	R-12 (75)
<i>Ovophis okinavensis</i>	R-4 (96), R-12 (72, 75, 97, 171, 182) M-5 (195)
<i>Ramphotyphlops braminus</i>	R-3 (49), R-4 (12), R-7 (170), R-8 (171), R-9 (171) M-5 (2, 189)
<i>Rhabdophis tigrinus tigrinus</i>	O-4 (29) A-1 (227) R-6 (13, 22, 44, 94, 145) V-3 (64), V-4 (39, 154*, 180, 209), V-5 (62, 89, 132), V-6 (36, 51, 52), V-11 (1,159), V-18 (37), V-21 (18, 85, 106, 133, 134, 140, 152, 153, 211), V-22 (194) M-10 (35)
<i>Trimeresurus elegans</i>	O-1 (220***) R-3 (168, 171) V-5 (167), V-12 (24, 88, 101, 149, 181, 223) M-4 (136, 138, 205)
<i>Trimeresurus flavoviridis</i>	R-4 (96, 169, 171), R-10 (118), R-12 (72, 75, 97) M-5 (207)
<i>Trimeresurus</i> sp.	M-15 (219)
Unidentified snake (Colubridae)	V-7 (51) M-2 (166), M-4 (136, 137, 205)
Unidentified snake	O-2 (141), O-5 (199) A-2 (176), A-4 (65) R-4 (44), R-6 (30), R-11 (11, 12), R-12 (10, 12) V-2 (93), V-4 (4, 9, 34, 39, 48, 79, 115, 121, 122, 126, 148, 154*,155, 156, 157, 158, 164, 174, 180, 183, 201, 204, 208, 212, 215, 216), V-5 (15, 41, 77, 78, 82, 83, 130, 185, 196), V-6 (36, 52, 60, 88, 102, 162), V-8 (203), V-10 (50), V-11 (112), V-12 (24, 40, 101, 128), V-13 (20, 21, 48, 53, 80, 81, 100, 124, 135, 216), V-15 (5), V-17 (150), V-18 (217), V-19 (90), V-20 (26), V-21 (27, 85, 106, 129, 222, 225) M-2 (47, 177, 213), M-4 (45, 139, 167), M-5 (2, 28, 46, 68, 125), M-6 (17), M-8 (142), M-9 (202), M-12 (184), M-13 (38, 173), M-14 (210), M-15 (219)

* This paper seems to include both original and cited data. Because it was not possible to distinguish them, we cited all of the data.

** Identification of the prey species is based on personal communication from the author.

*** The author stated that he had seen a record of an *Anguillia marmorata* that had swallowed a *Trimeresurus elegans*.

bles with Japanese common names will be published in the Bulletin of the Herpetological Society of Japan.

Predators of Japanese snakes revealed by the literature survey are listed in Table 1. Predators of each species or subspecies of snakes along with references are listed in Table 2. We confirmed 59 species/subspecies of predators, encompassing a range from invertebrate arachnids to large mammals. At least one predator was recorded for 21 species of snakes, which is approximately half of the Japanese terrestrial snakes. It should be noted that these tables are not to be considered an all-inclusive summary of the predators of Japanese snakes. We hope that this review will stimulate biologists and naturalists to observe and report further predatory events on snakes and promote future studies on defensive mechanisms of snakes.

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SUPPLEMENT

226. M. HASEGAWA. Personal Communication.
227. TOCHIMOTO, T. AND K. SHIMIZU. 2000. On the foods of the Japanese giant salamander *Andrias japonicus* (Temminck, 1837). (Oral presentation at the 39th Annual Meeting of the Herpetological Society of Japan)

APPENDIX

Predators of sea snakes

Predator—Chondrichthyes, Carcharhiniformes : *Galeocerdo cuvier*

Prey—*Emydocephalus ijimae* (App. 2); *Hydrophis melanocephalus* (App. 2); *Laticauda semifasciata* (App. 2); *Pelamis platurus* (App. 1); Unidentified sea snake (App. 2)

Literature

- App. 1. UCHIDA, K. 1979. *An Essay on the Natural History of Fishes*. Rippu Shobo, Tokyo. 242 p. (**)
- App. 2. YANO, K. 1998. *Sharks. Mysterious Ecology of the Chondrichthyes*. Tokai Univ. Press, Tokyo. 223 p. (**)

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Abstracts in Japanese will appear in
Bulletin of the Herpetological Society of
Japan, Vol. 2001, No. 1.

[Turtles]

1. Satellite tracking of male loggerhead turtle (*Caretta caretta*) migration. By: Naoki Kamezaki, Kojiro Mizuno, Masato Kobayashi, and Takaaki Nishi.
2. Distribution of nesting sites of sea turtles in the Okinawa Islands, Miyako Islands, and Yaeyama Islands. By: Koichi Hirate, Naoki Kamezaki, Akira Kikukawa, Tetsuya Kondo, Kenji Kuroyanagi, Keiichi Nomura, and Tatsuya Shima.
3. Seasonal changes of movement pattern in the yellow-margined box turtle (*Cistoclemmys flavomarginata evelynae*). By: Koreyuki Kurosawa and Masako Izuwa.
4. Phylogenetic relationships of the genus *Mauremys* (Testudines: Bataguridae). By: Masanao Honda, Yuichirou Yasukawa, and Hidetoshi Ota.
5. Taxonomic status of two Pleistocene fossil turtles, *Cuora miyatai* and *Clemmys yabei* (Reptilia: Bataguridae), from Kuzuu, Tochigi Prefecture. By: Yuichirou Yasukawa and Ren Hirayama.
6. Sexual dimorphism in the Chinese soft-shelled turtle, *Pelodiscus sinensis*. By: Hiroyuki Sato, Atsushi Kaneko, and Hidetoshi Ota.
7. Normal embryonic development of the Chinese soft-shelled turtle, *Pelodiscus sinensis*. By: Masayoshi Tokita and Shigeru Kuratani.
8. Distribution, food habitats, and reproductive cycles of four introduced freshwater turtles (*Trachemys scripta ele-*

gans, *Mauremys mutica*, *Chinemys reevesii*, and *Pelodiscus sinensis*) on Okinawa Island. By: Dai Hatanaka and Takeshi Sasaki.

[Lizards]

9. Phylogeny and biogeography of the agamid genus *Japalura* in East Asia. By: Hidetoshi Ota, Masanao Honda, Szu-Lung Chen, and Tsutomu Hikida.
10. Sexual size dimorphism of the agamid lizard *Japalura polygonata*. By: Satoshi Tanaka.
11. Distribution of the genus *Gekko* in Nagasaki Prefecture. By: Takanori Matsuo.
12. Phylogenetic relationships among the Japanese members of the genus *Gekko*. By: Mamoru Toda, Hidetoshi Ota, and Tsutomu Hikida.
13. Distribution of house geckos in Taiwan and Amamioshima Island of the Ryukyu Archipelago. By: Takahiko Kuze and Hidetoshi Ota.
14. Clonal composition of the parthenogenetic gecko, *Lepidodactylus lugubris*, in Taiwan. By: Saiko Yamashiro, Wen-hao Chou, and Hidetoshi Ota.
15. Taxonomic notes on specimens of the genus *Scincella* (Squamata: Scincidae) from Vietnam. By: Szu-Lung Chen, Nikolai L. Orlov, Ilya Darevsky, Hidetoshi Ota, and Tsutomu Hikida.
16. Three undescribed species of the flat-bodied water skinks of the genus *Tropidophorus* (Reptilia: Scincidae) from Thailand and Vietnam. By: Tsutomu Hikida, Hidetoshi Ota, Jarujin Nabhitabhata, and Nikolai L. Orlov.
17. Additional notes on reproduction of

Takydromus lizards of the Nansei Shoto.
By: Sen Takenaka and Hajime Moriguchi.

[Snakes]

18. Geographic morphological variation in a colubrid snake, *Dinodon semicarinatum*, from the central Ryukyus, Japan. By: Isao Takiguchi and Hidetoshi Ota.
19. Appearance rate of *Elaphe quadrivirgata* in an outdoor enclosure. By: Hajime Moriguchi.
20. Geographic variations in morphology of the two *Emydocephalus* species, *E. ijimae* and *E. annulatus*. By: Gen Masunaga and Hidetoshi Ota.
21. Food habits of sea snakes in the Ryukyu Archipelago. By: Mayuko Morishita, Gen Masunaga, and Hidetoshi Ota.
22. Geographic variation and population systematics of *Trimeresurus flavoviridis* and *T. tokarensis* in the central Ryukyus, Japan. By: Hiroko Nagatomo and Hidetoshi Ota.
23. Distribution and ecology of *Trimeresurus mucrosquamatus* established on Okinawa Island. By: Masahiko Nishimura and Hiroyuki Akamine.
24. A case of envenomation by the habu, *Trimeresurus flavoviridis*. By: Setsuko Iwanaga, Mamoru Toda, Tadafumi Maenosono, and Hidetoshi Ota.
25. Classification of *Agkistrodon* (sensu lato) and *Trimeresurus* (sensu lato) based on micro-ornamentation of scale surface. By: Michihisa Toriba.
26. Literature survey on predators of snakes in Japan. By: Koji Tanaka and Akira Mori

[Reptiles general]

27. General growth model and its examination by reptiles' growth data. By: Masami Hinoue.

[Salamanders]

28. On the prey of the Japanese giant salamander *Andrias japonicus* (Tem-

minck, 1837). By: Takeyoshi Tochimoto and Kunikazu Shimizu.

29. Population structure of the Japanese giant salamander, *Andrias japonicus*, in two streams of the Kannose River, Hiroshima Prefecture. By: Sumio Okada, Taeko Utsunomiya, Yasuaki Utsunomiya, and Jun-ichi Naito.
30. Site selection for breeding in lotic waterbodies by *Hynobius tokyoensis*. By: Sadao Ihara.
31. Winter and spring breeding in same population of *Hynobius lichenatus*. By: Hiroshi Ota.
32. Sex chromosomes in *Hynobius nigrescens*. By: Chikako Ikebe and Seiichi Kohno.
33. Ontogenetic development of hind limbs in *Hynobius kimurae*. By: Yasuchika Misawa and Masafumi Matsui.
34. Variation in external morphology of *Hynobius naevius* from Amagi City, Fukuoka Prefecture. By: Atsushi Tominaga, Masafumi Matsui, and Kanto Nishikawa.
35. Genetic variation among populations of *Hynobius boulengeri*. By: Kanto Nishikawa, Masafumi Matsui, Sin'ichi Sato.
36. Ecological comparison of two species of salamander distributed in Hokkaido, Japan. By: Takanori Sato, Shigehiro Nakabayashi, Nobuyuki Narumi, Takehito Ueda, and Yukihiro Kohmatsu.

[Frogs]

37. Differences in body size, growth, and age at maturity between two populations of the Japanese toad (*Bufo bufo formosus*). By: Ayako Ochi.
38. Influence on spawning sites of the Japanese Toads, *Bufo torrenticola* and *B. bufo formosus*, by a flood of the Nozumi River, Toyama Prefecture. By: Hisao Nambu, Tamotsu Fukuda, and Yukimasa Araki.
39. Molecular phylogeny of the Eurasian *Bufo bufo* group and allied groups. By:

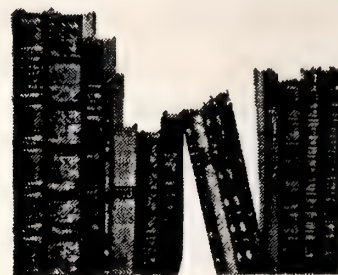
- Wanzhao Liu and Masafumi Matsui.
40. ζ -Crystallin expresses in the Japanese tree frog (*Hyla japonica*) lens as a taxon-specific crystallin. By: Hisashi Kimoto, Yutaka Fujii, Yuichirou Yasukawa, Keiko Ishikawa, Kikuko Watanabe, Akira Taketo, and Noboru Nakai.
 41. Genetic variation among populations of *Rana pirica*. By: Giedrius Trakimas, Masafumi Matsui, Kanto Nishikawa, and Kiyoshi Kasugai.
 42. Allometry of *Rana tagoi* from Shishigatani, Kyoto. By: Tadafumi Sugihara and Masafumi Matsui.
 43. Phylogenetic relationships among the brown frogs from Eurasia. By: Tomoko Tanaka-Ueno and Masafumi Matsui.
 44. A preliminary experiment on recognizing individuals of *Rana nigromaculata*. By: Takeshi Kameyama, Toshihiro Morita, and Sumio Okada.
 45. Age determination of the Daruma pond frog, *Rana porosa brevipoda*, by skeletochronology. By: Wichase Khonsue, Masafumi Matsui, and Yasuchika Misawa.
 46. Notes on hibernation of a Japanese pond frog, *Rana porosa brevipoda*. By: Toshihiro Morita, Takeshi Kameyama, and Sumio Okada.
 47. Examples of homing ability of *Rana catesbeiana*. By: Junsuke Marunouchi, Tamotsu Kusano, and Masayuki Sumida.
 48. Breeding of the ranid frog, *Rana narina*, on Okinawajima Island, Ryukyu Archipelago. By: Masanao Toyama.
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A New Book from the Society for the Study of Amphibians and Reptiles

SLITHY TOVES

ILLUSTRATED CLASSIC HERPETOLOGICAL BOOKS
AT THE UNIVERSITY OF KANSAS IN PICTURES
AND CONVERSATIONS by Sally Haines

Eighty-four Illustrations in Full Color



THIS BOOK IS IN THE FORM OF A GALLERY GUIDE TO A REMARKABLE EXHIBIT OF ILLUSTRATED HERPETOLOGICAL BOOKS DATING from the 16th to the 20th centuries. It results from a display arranged by the Kenneth Spencer Research Library of the University of Kansas on the occasion of the 1996 SSAR meeting held at the university. Books such as these, many of which have exquisitely handcolored illustrations by some of the greatest natural history artists, are today kept under lock and key in institutional libraries and therefore are scarcely known to most herpetologists, but the enthusiasm of the SSAR conference goes for the Kansas exhibit has encouraged us to make this printed version available. The author, Sally Haines, Associate Special Collections Librarian at the Spencer Library, is in charge of the natural history collections.

The bulk of this volume consists of full-color reproductions of illustrations from classic works together with extensive captions containing information about the authors, the artists, and the books. It is an iconography of herpetology and an essential part of the history of the field.

Organization of the Book

Myth versus Reality: the Challenge of Illustrating Amphibians and Reptiles, *by Kraig Adler*
Part I — Herpetology to 1758: The Pre-Linnaean Period
Part II — Herpetology after 1758: Beyond Linnaeus
Part III — West of Eden: New World Herps
Part IV — The Seven Deadly Sins and other Evils: Herps as Symbol
References to Historical and Biographical Works
List of All Illustrated Herpetological Works in the Spencer Library
Chronological Index to Illustrated Herpetological Works in the Spencer Library

Some of the earliest books represented in this volume are by Pliny, Abbatius, Topsell, Seba, Linnaeus, and Rösel von Rosenhof, with later ones by Daudin, Rüppell, Bonaparte, Schlegel, and Fayrer, among many others. There is a special section on books about the Western Hemisphere, including works by Sloane, Catesby, Bartram, Spix, Holbrook, Cuvier, and Agassiz. The final section, on herps as symbol, includes religious manuscripts, sea serpents, astronomical atlases, and even illustrations from the works of John Milton, Mark Twain, and Walt Kelly. Included is a detailed listing of all illustrated herpetological works in the Spencer Library, which holds one of the great collections of natural history books. This volume represents a veritable treasure trove of some of the finest illustrations of amphibians and reptiles ever produced in book form and extensive bibliographic information about them.

Specifications: 190 pages, 8½ × 11 inches (21.5 × 28 cm), full color throughout (84 illustrations). Softcover. ISBN 0-916984-53-2. To be published December 2000.

PRICES: Pre-publication price to SSAR members US\$50; Institutions and non-members US\$60.

SHIPPING COST: USA address, add US\$3; non-USA address, add US\$6

SEND ORDERS TO: Dr. Robert D. Aldridge, SSAR Publications Secretary, Department of Biology, Saint Louis University, 3507 Laclede Avenue, Saint Louis, Missouri 63103-2010, USA (*telephone:* area code 314, 977-3916 or -1710; *fax:* 314, 977-3658; *e-mail:* ssar@slu.edu). Please make checks payable to "SSAR." Overseas orders must be paid in USA funds using a draft drawn on American banks or by International Money Order. Orders may also be charged to MasterCard or VISA (please provide the account number and card expiration date). SSAR membership information and a complete list of all Society publications can be obtained on request to Dr. Aldridge.

A New Book from the Society for the Study of Amphibians and Reptiles

THE HERPETOFAUNA OF NEW CALEDONIA

by AARON M. BAUER *and* ROSS A. SADLIER

with French summaries by Ivan Ineich and 189 color photographs

NEW CALEDONIA, INCLUDING THE LOYALTY ISLANDS and an associated group of smaller islands and reefs, is a French territory located in the tropical Southwest Pacific equidistant from New Guinea, New Zealand, and Australia. This ancient group of islands supports one of the most highly endemic and species-rich herpetofaunas in the Pacific region. Among the 71 species of terrestrial reptiles, 86% are endemics, and most belong to endemic genera. Despite being only 2.5% the size of New Guinea, New Caledonia has 36% as many lizard species. In addition, the New Caledonian barrier reef system, one of the largest and most diverse in the world, is inhabited by a dozen species of seasnakes. Because of the diversity of its flora and fauna and the fragility of its habitats, New Caledonia is regarded as a biodiversity “hot spot,” one of the earth’s biologically richest and most endangered terrestrial ecoregions.

This book—at the same time a scientific monograph and a field guide—is the first modern review of the amphibians and reptiles of New Caledonia. It covers the frogs, family Hylidae (1 species), geckos of the families Diplodactylidae (20) and Gekkonidae (6), the Scincidae (42), snakes of the families Boidae (1), Elapidae (12, all marine), and Typhlopidae (2), and the sea turtles, Cheloniidae (3). Geckos and skinks, in fact, are the most numerous and dominant terrestrial vertebrates in New Caledonia. These two groups have undergone extensive generic and specific diversification, including the world’s largest living geckos (*Rhacodactylus*) and more than a dozen genera of skinks including the giant skinks (*Phoboscincus*).

The authors, Aaron M. Bauer (USA) and Ross A. Sadlier (Australia) are both noted authorities on the Pacific herpetofauna. Their extensive field work in New Caledonia began more than 20 years ago. As a result of their research, numerous new genera and species of New Caledonian geckos and skinks have been described and named, but this book represents the first synthesis of their 20 years of study.

Organization of the Book

- Geography, Vegetation, Geological History, and Biogeography
- Ecological Patterns of Terrestrial and Marine Species
- Conservation
- Humans and the Herpetofauna, including a History of Scientific Studies
- Systematic Accounts of Genera and Species: Keys; Synonymies; Descriptions; Distribution (spot map for each species); Natural History; Conservation Status; Remarks and Status
- Incidental Taxa, Taxa of Questionable Occurrence, and Those Erroneously Recorded from New Caledonia
- Fossil and Subfossil Species
- Literature Cited and Bibliography of New Caledonian Herpetology (more than 1000 references)
- Gazetteer of Place Names; Index to Scientific Names

The 153 color photographs of animals depict nearly every species. There are also 36 photographs of New Caledonian habitats.

Specifications: 325 pages, 7 × 10 inches (18 × 22.5 cm), plus 189 color photographs of animals and habitats on 24 plates, 47 maps, 63 figures, and 4 tables. Clothbound. ISBN: 0-916984-55-9. To be published December 2000.

PRICES: Pre-publication price to SSAR members US\$50; Institutions and non-members US\$60.

SHIPPING: USA address, add US\$3; non-USA address, add US\$6.

SEND ORDERS TO: Dr. Robert D. Aldridge, SSAR Publications Secretary, Department of Biology, Saint Louis University, 3507 Laclede Avenue, Saint Louis, Missouri 63103-2010, USA (*telephone:* area code 314, 977-3916 or -1710; *fax:* 314, 977-3658; *e-mail:* ssar@slu.edu). Please make checks payable to “SSAR.” Overseas orders must be paid in USA funds using a draft drawn on American banks or by International Money Order. Orders may also be charged to MasterCard or VISA (please provide the account number and card expiration date). SSAR membership information and a complete list of all Society publications can be obtained on request to Dr. Aldridge.



Sri Lanka: December, 2001

WORLD CONGRESS OF HERPETOLOGY

We are happy to inform you that the Fourth World Congress of Herpetology will be held from 2nd to 9th December 2001, in the BMICH, Colombo Sri Lanka. This is the first significant herpetology event of the new millennium and the organizing committee is determined to make this the most memorable herpetological forum of the century.

The Registration—Full participant US \$ 350, Students US \$ 250, and Accompany persons US \$ 200. A surcharge of US \$ 100 will be applied after 1st September 2001.

Deadline for abstracts—31 August 2001.

Registration entitlements for delegates—In addition to attendance at all formal sessions, congress registration will be entitle you to:

Airport transfers (both ways)

Daily shuttle service from hotel to congress venue

Conference kit, which includes: Label badge, Special 4WCH pen, Special 4WCH note pad, 4WCH picture post cards, Sri Lanka herp stickers, Welcome cocktail, Two coffee breaks with snacks on each day of the congress, Lunch on all congress days, Congress banquet, Full day professional excursion, Abstract book and the programme and many more.

We have reserved special discount rate in leading five star hotel — shared room with breakfast is only US \$ 35 on wards.

We will be mailing you the congress Brochure and Registrations forms.

Sri Lanka is a herpetological hotspot in the world. In addition to upward 150 species of amphibians, Sri Lanka is also home to diverse reptiles: Five species of marine turtles, chelonians, crocodiles, varanids, agamids, skinks, lacertids, chameleons, geckos, and snakes (from primitive uropeltids to modern vipers) inhabit our small island of 64,742 square km in area.

I look forward to welcoming you in Sri Lanka in December 2001.

REGISTER TODAY!

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Manuscripts in English should be typed on sturdy typing paper of A4 size (28 × 21 cm). Typing should be double-spaced with 2.5 cm margins on all sides. Words should not be broken at the end of a line. Full papers should not exceed 12 printed pages, and short communications should not exceed 2 printed pages. Longer papers may be printed, on condition that the author pays the excess page charges in full. Each of the following divisions should be begun on a separate page: cover page, title page, abstract, main text, references, each table, figure legends, each figure. The cover page should show the title, name of author and addresses to whom communications should be directed with his or her telephone and fax numbers, and E-mail address, the date of submission, the number of pages of main text, the number of figure legends, number of figures, and number of tables.

The title page should show the title, the name(s) of the author(s), and their affiliation(s) and addresses. In the case of several authors, indicate the one to whom communications should be directed, his or her telephone and fax numbers, and E-mail address. The abstract page should contain an abstract of about 200 words and five keywords. The main text should consist of an Introduction, Materials and Methods, Results, Discussion, and Acknowledgements. Format for text, citations, and literature cited should follow those used in the most recent issue of the journal. Short reports should follow the format of full papers, including the use of subtitles for Materials and Methods, etc. Each table should be on a separate page. Line drawings should be such that they can be used directly for printing. Figures larger than 28 × 21 cm cannot be accepted. When several drawings or photographs are to be reproduced as one figure, they should be mounted on cardboard in the desired arrangement. Figure legends should be collectively typed on a separate page.

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FUTURE MEETING

Niigata University, Niigata, Japan, November 2001
(Kunio Sekiya, Chair)

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